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REPORT

OF THE

SOIL CHEMIST AND BACTERIOLOGIST

OF THE

New Jersey Agricultural College Experiment Station

New Brunswick, N. J.

BY

JACOB G. LIPMAN

For the Year 1909

TRENTON, N. J.
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REPORT OF THE SOIL CHEMIST AND BACTERIOLOGIST.

JACOB G. LIPMAN.

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EXPERIMENTS ON AMMONIA AND NITRATE FORMATION IN SOILS.

It is now conceded by all students of soils that bacteria are one of the essential factors in crop production. It is generally recognized that the micro-organisms in the soil are concerned in the transformation of nitrogen compounds, as well as in the building of new nitrogenous substances out of elementary atmospheric nitrogen. It is recognized, just as widely, that bacteria are necessary for the proper circulation of nearly all of the plant-food constituents in cultivated soils. But while recognizing the importance of bacteria in supplying available plant-food to crops, investigators of soil fertility problems find themselves unable, at times, to measure quantitatively the progress of bacteriological changes; this is especially true of certain large groups of soil bacteria prominent in the decomposition of crop residues and of animal manures applied to the land.

As we look over the list of methods suitable for the quantitative study of bacteriological reactions, we find it far from satisfactory. The methods available are few, and are not always as reliable as one might wish them to be. We have at our disposal the plate method, whereby we can count the number of bacteria present in the soil at any given time; or, to be more correct, we can count *some* of the bacteria present in the soil. The limitation of this method need scarcely be dwelt upon here. Only a portion

(sometimes a small portion) of the bacteria present in the soil will appear on the plates; and even if all of them grew and formed colonies we should not be able to use mere numbers as a reliable guide in the study of soil fertility.

A greater measure of success may be attained not by counting the bacteria themselves, but by determining the extent of chemical change produced by them under given conditions. We may thus measure the rate of oxidation of carbon in the soil, either by determining the amount of carbon dioxide evolved, or the amount of oxygen absorbed; this method has not been used as extensively as it will be used in the future. It is safe to predict that it will furnish many important data and will help to explain facts that are now obscure.

Other methods for measuring the activities of soil bacteria concern not the carbon, but the nitrogen of organic materials. Knowing that the protein compounds in the latter are gradually split into albumoses, peptones, amino-acids and their derivatives, and ammonia; and knowing, further, that the last named is oxidized to nitrites and nitrates we have attempted to estimate quantitatively some of the cleavage products, hoping thereby to gain an insight into the relations existing among several groups of soil bacteria. The earlier work in this direction consisted of determinations of ammonia, nitrites and nitrates present in the soil at different times and under different methods of treatment. It was soon realized, however, that the first two are only transition products, that they do not accumulate in the soil to any appreciable extent, and that they cannot be used, therefore, as a measure of oxidation processes in the soil. The determination of nitrates, on the other hand, gives more satisfactory returns, for it is the end product of oxidation and as such tends to accumulate in the soil; hence, valuable information has been secured by systematic study of nitrate formation, and its relation to crop growth. To be sure, also, this method has its limitations. Fairly stable as they are, nitrates may be used up by various micro-organisms; they may be leached out by heavy rains, or they may be taken up by crops growing on the land. For these reasons the determinations of nitrates in soil occupied by crops is not calculated to give adequate information concerning the rate of decomposition of organic substances. In uncropped soils, on the other hand, the systematic determination

of nitrates is decidedly more valuable as a measure of the activities of, at least, the nitrifying bacteria.

Now, it happens that nitrate formation in the soil is regulated more or less directly by preceding ammonia formation. The latter in its turn is dependent upon the number, kinds and vigor of the decay bacteria. It will be readily seen, therefore, that in soils where ammonia formation is feeble nitrification cannot be very extensive, even though the nitrite and nitrate organisms be active and numerous. But that is not all—apart from the groups of bacteria just mentioned we have in the soil other groups important in the transformation of nitrogen compounds. We have the so-called denitrifying bacteria capable of reducing nitrates more or less completely; we have organisms like those of the urea group capable of causing intensive changes in certain amino-compounds; we have various nitrogen fixing bacteria; and we have others whose activities are hardly recognized.

As we learned to know more about the specific chemical reactions caused by certain kinds of soil bacteria we began to look about for methods that would enable us to measure, with some accuracy, the extent of the chemical changes produced. The first systematic attempt to measure the activities of different groups of soil bacteria was made by Remy[1]. He proposed the use of peptone solution for measuring the activities of ammonia-producing bacteria; of ammonium sulphate and nitrite solutions for measuring the activities of nitrous and nitric bacteria, respectively; of nitrate solutions for measuring the activities of denitrifying bacteria, and of mannite solutions for measuring the activities of nitrogen-fixing bacteria. In each case such other substances were added to the nutrient solutions as were calculated to favor the development of the particular group of bacteria.

It would not be advisable to review here the rather considerable number of papers bearing on the value of Remy's methods[2]. In their original form their use is not profitable even though they may indicate, occasionally, certain chemical and bacteriological differences in soils. It is believed, however, that modifications of these methods, or new methods based on the same principle, may

[1] Centbl. Bakt. (&c.), 2 Abt. 8 (1902), pp. 657, 699, 728, 761.

[2] See Bull. 194, Office of Experiment Stations, U. S. Department of Agriculture; also Reports of the Soil Chemist and Bacteriologist, New Jersey Experiment Stations, 1905, 1906, 1907 and 1908.

be utilized to great advantage in defining numerous reactions in the soil, that cannot be made clear by purely chemical methods. The data presented in the following pages comprise the study of methods concerning ammonia formation in soils, and culture solutions, as well as a number of facts bearing a more general relation to the processes of ammonification and nitrification.

BACTERIOLOGICAL RELATIONS AS AFFECTED BY ADDITIONS OF DEXTROSE AND SODIUM CITRATE TO THE SOIL.

The material for this experiment was secured from a fertile field that has been receiving, annually, applications of manure and fertilizer. A quantity of surface soil from this field was carefully sifted and portions of it weighing twenty-two pounds each were placed in large earthenware pots. A glazed earthenware saucer was placed under each of these pots and the required amounts of water were poured from time to time into these saucers so as to ensure a uniform moisture content in the several soils. There were in all eight pots in the experiment, numbered from 1 to 8. The treatment of the eight soils in question was as follows:

1 and 2	nothing.	
3 " 4	22.5 gms.	dextrose.
5 " 6	45.0 "	"
7 " 8	45.0 "	sodium citrate.

The dextrose and sodium citrate were carefully mixed with the soil in each case, and an attempt was made thereafter to maintain the moisture content of the soils at about 12.5 per cent. Samples were drawn at more or less regular intervals and ammonification tests were made by the Remy method and also by the beaker method to be described later. In addition to these, counts were made on agar plates of the bacteria present in the soils at different times, and determinations were made, likewise, of the amounts of nitrate present in the soil samples. Quantities of soil equivalent to those withdrawn in the samples were replaced from other pots treated in the same way. The experiment was begun on November 25th. Samples for bacteriological study were drawn on December 15th, January 4th, January 21st and February 11th.

The solid medium used for plating was made up as follows:

1000	c.c. tap water.
10	gms. dextrose.
0.5	" K_2HPO_4.
0.2	" $MgSO_4$.
0.05	" peptone.
20.0	" agar.

As shown by the colonies on the agar plates the following numbers of bacteria were present in one gram of each of the different soils:

Pot No.	*Dec. 15, 1908, Average.*	*Jan. 4, 1909, Average.*
1	7,000,000	4,760,000
2	8,400,000– 7,700,000	5,060,000– 4,910,000
3	52,000,000	10,600,000
4	7,100,000– 6,750,000	4,640,000– 4,500,000
5	70,000,000	19,000,000
6	80,000,000–75,000,000	21,000,000–20,000,000
7	Too numerous.	100,000,000
8	" "	60,000,000–80,000,000

Pot No.	*Jan. 21, 1909, Average.*	*Feb. 11, 1909, Average.*
1	3,600,000	3,040,000
2	3,980,000– 3,790,000	3,400,000– 3,220,000
3	6,400,000	4,360,000
4	7,100,000– 6,750,000	4,640,000– 4,500,000
5	19,200,000	9,000,000
6	15,600,000–17,400,000	7,840,000– 8,400,000
7	48,000,000	15,040,000
8	60,000,000–54,000,000	18,000,000–16,520,000

The data just presented show the enormous increase of bacteria in the soil brought about by the addition of dextrose and sodium citrate. On December 15th, that is about three weeks after the beginning of the experiment, the untreated soils contained, on the average, 7,700,000 bacteria per gram. The soils which received the smaller application of dextrose contained, on the average, 57,500,000 bacteria per gram; while those which received the larger application contained, on the average, 75,000,000 per gram. Soils 7 and 8 which received additions of sodium citrate contained a number of bacteria so large that a quantity of soil weighing 0.005 mg. caused the development of colonies on the plates too numerous to count.

The plates prepared subsequently showed essentially the same relations. The dextrose treated soils maintained throughout their prominence as to the number of bacteria present in them; while the sodium citrate treated soils contained even greater numbers of micro-organisms than were present in the other soils. We note, however, that there was a gradual decline in numbers. On February 11th, the untreated soils contained only 3,220,000 bacteria per gram; while the treated soils showed even more striking a decrease in the number of their bacteria than the untreated soils. We may note, particularly, that in soils 3 and 4 there was a decrease in less than three months from 57,500,000 bacteria per gram to 4,500,000 per gram. The decrease in soils 5, 6, 7 and 8 was even more pronounced. This tendency towards the decline in the numbers of bacteria in greenhouse soils was noted also in other experiments published elsewhere. It seems that the thorough æration of the soil, favored by sifting and filling into pots, encouraged bacterial growth and intense oxidation of the humus and of other organic substances. In the course of time, however, the readily oxidizable substances disappeared and with them many of the decay bacteria.

The varying numbers of bacteria as noted above should, theoretically, at least, bear a more or less direct relation to the amounts of ammonia produced from the soil humus. The Remy method attempts to measure this relation by introducing equivalent quantities of soil into sterile portions of a peptone solution, and determining the amounts of ammonia formed in a given time. The usual procedure consists of adding ten gms. of soil, or of an equivalent quantity of soil infusion, to 100 c.c. of a sterile solution of peptone, and of determining the amount of ammonia produced at the end of three or four days.

Soils 1–8 were employed for inoculating peptone solutions in the manner just indicated, on December 15th, January 4th, January 21st and February 11th, that is, at the same time that the agar plates were prepared from the corresponding samples. The amounts of ammonia nitrogen found on the different dates were as follows:

AMMONIA NITROGEN IN THE PEPTONE SOLUTION.

Soil No.	Dec. 18—Aver. mg.	Jan. 7—Aver. mg.	Jan. 25—Aver. mg.	Feb. 15—Aver. mg.
1	89.45	75.72	22.21	7.97
1	86.99– 88.22	86.75–81.23	23.67–22.94	7.97– 7.97
2	89.31	80.59	22.86	8.93
2	88.73– 89.02	80.59–80.59	23.19–23.02	8.77– 8.85
3	90.32	79.94	22.70	10.04
3	89.02– 89.67	82.73–81.33	21.24–21.97	10.36–10.20
4	97.70	79.12	21.24	11.16
4	100.45– 99.07	77.67–78.39	21.08–21.16	11.32–11.24
5	86.27	78.32	27.08	10.20
5	83.23– 84.75	89.73–84.02	28.53–27.80	10.68–10.44
6	83.52	72.80	26.59	9.72
6	86.27– 84.89	84.48–78.64	27.73–27.16	11.00–10.36
7	103.35	93.01	35.02	13.23
7	105.38–104.36	99.56–96.28	36.32–35.67	14.19–13.71
8	102.05	95.18	33.89	15.62
8	Lost–102.05	90.90–93.04	31.78–32.83	15.46–15.54

Ammonia production in the peptone solutions failed to show a direct relation to the numbers of bacteria found to have been present in the soils under consideration. Exception may be made only in the case of soils 7 and 8 where sodium citrate had been employed. The enormous number of bacteria in these soils seems to have favored more intense ammonification in the peptone solutions; in other instances, however, the relation between numbers and ammonia production is not always apparent. Thus in soil 2 there were present on December 15th, 8,400,000 bacteria per gram; and in soil 3, 52,000,000 per gram. When these soils were used for inoculation into peptone solutions they gave rise to the formation of 89.02 mg. and 89.67 mg., respectively, of ammonia nitrogen. Other instances of this sort may be readily discovered on examining the table given above. It should only be added here that the agreement between duplicate portions of the peptone solutions inoculated with the same soil is not entirely satisfactory in a number of cases.

Everything considered, therefore, the Remy method failed to yield consistent results in the present experiments, even though in some instances it indicated differences in the ammonifying power of the several soils. Admitting that ammonia production and numbers of bacteria need not go together, and that proper allowance should be made for variations in species and physiological efficiency, the results secured still remain unsatisfactory because of

the lack of better agreement, in several instances, in the duplicate inoculations. This lack of agreement could hardly be attributed to insufficient care in the sterilization of the peptone solutions, or to the method or inoculation. All of the solutions were sterilized in the autoclave and were inoculated, when cool, with equal portions of carefully prepared infusions from the corresponding soils.

Simultaneously with the inoculation of the peptone solutions 100 gm. quantities of soil, taken from the several pots, were each thoroughly mixed with 0.5 gm. of peptone. At the end of three or four days these portions of soil kept in covered beakers and maintained at the same moisture content by means of sterile water, were transferred to copper flasks and the ammonia was distilled off and determined. The amounts of ammonia nitrogen found were as follows:

AMMONIA NITROGEN IN THE 100 GM. PORTIONS OF SOIL.

Soil.	*No.*	*Dec. 18.* mg.	*Aver.* mg.	*Jan. 7.* mg.	*Aver.* mg.	*Jan. 25 & 26.* mg.	*Aver.* mg.
1	.....	49.21		44.99		47.19	
1	.....	50.37	49.79	49.69	47.34	51.24	49.21
2	.....	49.21		46.61		47.80	
2	.....	51.53	50.37	49.69	48.15	51.40	49.60
3	.....	46.17		43.05		47.80	
3	.....	48.49	47.33	45.16	44.10	49.62	48.71
4	.....	48.49		45.80		44.91	
4	.....	48.92	48.70	45.48	45.64	48.97	46.94
5	.....	47.33		45.32		45.56	
5	.....	46.32	46.82	47.58	46.45	47.80	46.68
6	.....	46.75		44.83		45.77	
6	.....	46.32	46.53	47.91	46.37	48.81	47.29
7	.....	48.78		45.31		45.73	
7	.....	48.49	48.63	47.91	46.61	46.70	46.21
8	.....	*41.98		45.64		44.59	
8	.....	48.20	48.20	48.07	46.85	46.70	45.64

The figures given above fail to show a direct relation between numbers and ammonia formation in soils. The duplicates show satisfactory agreement in most cases, and soils 1 and 2 seem to have caused the production of more ammonia than the other soils. Otherwise the yields of ammonia nitrogen differed but little. It will be remembered that in the peptone solutions, soils 7 and 8, which received additions of sodium citrate, produced more ammonia than any of the other soils; in the 100 gm. portions of soil

* Not included in the average.

these relations were not apparent. It should be added here that in one series of soils started January 21st, the ammonia determinations were made in one set on January 25th, and in the duplicates on January 26th; for this reason the duplicate determinations contain somewhat larger amounts of ammonia nitrogen.

Taking the results as they stand, we must assume that in the peptone solutions the comparatively small numbers of bacteria introduced with soils 1 and 2 produced as much, or nearly as much, ammonia as was produced by the much larger numbers introduced with the other soils. Such being the case, no greater differences could have been expected in the soils themselves, where the 100 gm. quantities contained an initial number of bacteria ten times as great in each case. Subsequent experiments reported in the following pages serve to throw a clearer light on the results in question.

Before passing on to other experiments on ammonia formation in soils, we may consider, briefly, the effect of the treatment on nitrate formation. The quantities of nitrate nitrogen found on the different dates were as follows when expressed in parts per million of soil:

NITRATE NITROGEN IN PARTS PER MILLION.

Soil No.	*Dec. 15, '08.*	*Jan. 4, '09.*	*Jan. 21, '09.*	*Feb. 11, '09.*
1	10.55	49.39	100.00	46.33
2	11.40	36.36	80.40	50.00
3	Trace	7.57	12.50	9.29
4	"	8.93	15.38	9.80
5	"	9.16	14.28	8.43
6	"	4.67	7.81	8.33
7	4.16	19.03	41.66	50.00
8	1.42	6.41	8.25	21.35

The influence of the soil treatment is apparent in the nitrate formation, as it had occurred in the soils under experiment. In the untreated soils 1 and 2, there was a gradual accumulation of nitrate until January 21st. Between that date, however, and February 11th there was a marked decrease, probably on account of the using up of the nitrate nitrogen by algæ and bacteria. There was a similar increase and decrease in soils 3, 4 and 5. In soils 6, 7 and 8, on the other hand, the increase was uninterrupted. We may observe, also, that on December 15th, that is about three weeks after the beginning of the experiment, the largest amount

of nitrate was found in the untreated soils. In all of the soils which had received additions of dextrose there were at that time only traces of nitrate nitrogen. On January 4th, there was not only a considerable increase of nitrate nitrogen in the untreated soils, but an accumulation of it also in the other soils. The accumulation of nitrate nitrogen was still greater on January 21st; nevertheless, the depressing effect of the dextrose was felt even on February 11th. On that date soils 3 and 4 contained somewhat more nitrate nitrogen than was present in soils 5 and 6. In the case of the sodium citrate, on the contrary, the depressing effect began to disappear after January 21st.

Mention may likewise be made here of the very considerable differences in the duplicate soils; for instance, in soil 6 the amounts of nitrate nitrogen were markedly smaller than those in soil 5. Similarly in soil 7 there was throughout a much larger amount of nitrate nitrogen than in the duplicate soil 8. The greatest difference appeared on January 21st when soil 7 contained about five times as much nitrate nitrogen as soil 8; we see, thus, that even with identical treatment the bacteriological changes in the two equal portions of soil may vary considerably. There seems to be a tendency, in the course of time, towards the establishment of uniformity in the duplicate portions, as may be seen by comparing the data for January 21st and February 11th, respectively.

In addition to the analytical data reported in the preceding tables, determinations were made also of the amounts of ammonia nitrogen present in the soils in the pots. These determinations showed very slight accumulation of ammonia even where dextrose was added, hence they need not be reported here.

AMMONIFICATION OF NITROGENOUS MATERIALS IN FERTILE SOIL AND SAND.

The experiments reported in the preceding pages demonstrated that the soil treatment affected the numbers of bacteria, and the formation of nitrates. The methods employed were capable of measuring with a fair degree of accuracy the differences in numbers and nitrate formation; on the contrary, the methods employed for the study of ammonia formation were not as satisfactory. It

was deemed advisable, therefore, to institute a number of other experiments that would throw more light on the nature of ammonia formation in soils.

The experiments in question were carried out with weighed quantities of soil kept under known moisture conditions in covered beakers. These soils received additions of nitrogenous materials capable of undergoing ammonification. When ammonia had accumulated in sufficient quantity it was distilled off and measured.

SERIES A.

This series included twelve portions of soil, each weighing 100 gms., and twelve portions of white sand also weighing 100 gms. each. The treatment was as follows:

Sample No.		Additions.
Soil.	*Sand.*	
1, 2	13, 14	Nothing.
3, 4	15, 16	0.5 gm. peptone.
5, 6	17, 18	1.0 " "
7, 8	19, 20	1.0 " egg albumen.
9, 10	21, 22	1.0 " dried blood.
11, 12	23, 24	0.25 " urea.

Soil portions 3, 5, 7, 9, 11, 15, 17, 19, 21 and 23 received additions of nitrogenous materials previously sterilized.

Soil portions 4, 6, 8, 10, 12, 16, 18, 20, 22, 24 received similar additions not previously sterilized.

Sterile water was added to the different soil portions in order to assure optimum conditions for bacterial growth. Ammonia determinations were made in portions 1, 3, 4, 5, 6, 11, 12, 13, 15, 16, 17, 18, 23 and 24 in four days, and in the others in eight days. A longer period was thus allowed for the formation of ammonia from the more inert albumen and dried blood. The amounts of ammonia nitrogen found were as follows:

Soil No.		*Soil No.*		*Average.*
1.....	1.13 mg.	2.....	0.81 mg.	0.97 mg.
3.....	48.48 "	4.....	45.56 "	47.02 "
5.....	74.91 "	6.....	93.39 "	84.15 "
7.....	77.51 "	8.....	71.51 "	74.51 "
9.....	19.13 "	10.....	18.15 "	37.28 "
11.....	55.46 "	12.....	36.65 "	46.05 "
13.....	0.16 "	14.....	0.32 "	0.24 "
15.....	7.46 "	16.....	7.78 "	7.62 "
17.....	13.78 "	18.....	10.70 "	12.24 "
19.....	8.12 "	20.....	6.37 "	7.24 "
21.....	5.89 "	22.....	8.28 "	7.08 "
23.....	9.57 "	24.....	7.29 "	8.43 "

The data as arranged in the above table show that in the white sand the decomposition of the nitrogenous materials proceeded more slowly than in the fertile soil. They show also that the yields of ammonia nitrogen from peptone, albumen, dried blood and urea were markedly different, at least in the fertile soil; in the white sand the small number of bacteria and the lack of uniformity in the species relationships did not allow these differences to be brought out as prominently. The previous heating of the several materials apparently affected the rate of ammonification, and the lack of agreement between duplicates, in some instances, may partly be ascribed to this cause. There is one other point to be noted in connection with the figures just presented, namely, that concerning the greater yields of ammonia from the 1.0 gm. quantities of peptone as compared with those from the 0.5 gm. quantities. There seemed to be here a certain rough relation between the amount of peptone used and the amount of ammonia formed. This relation was made, therefore, the basis of further experiments.

AMMONIA PRODUCTION IN THE SOIL FROM DIFFERENT AMOUNTS OF PEPTONE.

SERIES B.

Varying amounts of peptone were mixed with 100 gm. quantities of fertile soil, which was maintained at a moisture content of about 18 per cent. The amounts of peptone employed were as follows:

Soil No.		
1, 2	0.5 gm. peptone.	Kept in incubator at 28° C. for 2 to 3 days.
3, 4	1.0 " "	
5, 6	2.0 " "	
7, 8	3.0 " "	
9, 10	0.5 " "	Kept at room temperature for 4 to 6 days.
11, 12	1.0 " "	
13, 14	2.0 " "	
15, 16	3.0 " "	

At the end of the stated intervals the soils were transferred to copper flasks and the ammonia was distilled off and determined in the usual way. The amounts of ammonia nitrogen found were as follows:

Soil No.	*In 2 days.*	*Soil No.*	*In 3 days.*	*Average.*	
1	53.19 mg.	2	52.55 mg.	52.87 mg.	At 28° C.
3	91.41 "	4	100.49 "	95.95 "	
5	158.45 "	6	171.99 "	165.22 "	
7	163.87 "	8	210.21 "	187.04 "	

Soil No.	*In 4 days.*	*Soil No.*	*In 6 days.*	*Average.*	
9	43.68 mg.	10	49.81 mg.	46.74 mg.	At room temperature.
11	76.03 "	12	88.61 "	82.32 "	
13	106.00 "	14	121.17 "	113.58 "	
15	107.28 "	16	125.33 "	116.30 "	

Soil portions 1, 3, 5 and 7 kept at 28° C. for two days yielded gradually increasing quantities of ammonia nitrogen as the amount of peptone used was increased. Thus in soil portion (1) with the 0.5 gm. of peptone there were formed 53.19 mg. of ammonia nitrogen; whereas in soil portion (2) with 1.0 gm. of peptone there were formed 91.41 mg. As the amounts of peptone used grew larger the absolute quantities of ammonia produced also increased, although not directly in proportion to the peptone employed.

In three days at 28° C. the quantities of ammonia formed were larger than those produced in two days. The differences grew greater with the increasing amounts of peptone, as may be seen readily by comparing the figures presented in the above table. The same relations may be observed also in the soil portions that had been kept at room temperature. Referring the data just noted to field conditions we may properly ask whether parallel results may not be secured from large or small applications of nitrogenous materials. The extensive data secured in plot and pot experiments would justify us in answering this question in the affirmative.

AMMONIA PRODUCTION IN THE SOIL FROM DIFFERENT AMOUNTS OF DRIED BLOOD.

SERIES C.

In order to determine whether similar relations would obtain with other nitrogenous materials a number of 100 gm. portions of soil were mixed with varying quantities of sterile, finely ground dried blood. The additions were as follows:

1, 2, 3, 4	10	gm.	dried blood.
5, 6, 7, 8	20	"	" "
9, 10, 11, 12	30	"	" "
13, 14, 15, 16	40	"	" "

Soil portions 1–4 after the addition of the dried blood and of ten c.c. of sterile water were found to contain about 14.5 per cent. of moisture; in order to compensate for the increased amounts of dried blood the other soil portions received further additions of water at the rate of 1.7 c.c. for each ten gm. of dried blood. It will be shown presently that these additions were not sufficient for creating optimum conditions for bacterial growth.

The several soil portions were kept at room temperature ranging from 65–75° F. Ammonia determinations were made in parallel portions at the end of five, seven, nine and twelve days, respectively. The amounts of ammonia nitrogen found on the different dates are given in the following table:

Soil No. In 5 days.	*Soil No. In 7 days.*	*Soil No. In 9 days.*	*Soil No. In 12 days.*
1.... 27.62 mg.	2....109.20 mg.	3....189.53 mg.	4....190.32 mg.
5.... 33.69 "	6.... 93.87 "	7....128.64 "	8....307.64 "
9.... 55.56 "	10....129.32 "	11.... 82.57 "	12....147.76 "
13.... 68.33 "	14.... 79.67 "	15.... 74.44 "	16....111.90 "

The ammonia determinations at the end of five days showed clearly the influence of the amount of dried blood employed. The determinations at the subsequent dates failed to bring out the same relations as clearly because of the insufficient amount of moisture. This applies particularly to the soil portions which had received the larger additions of dried blood.

AMMONIA PRODUCTION FROM VARYING AMOUNTS OF PEPTONE, DRIED BLOOD AND COTTONSEED MEAL IN VARYING QUANTITIES OF SOIL.

SERIES D.

In the present series varying amounts of dried blood were again used, as well as varying amounts of cottonseed meal and peptone. Additional quantities of moisture were supplied in proportion to the organic matter used, an attempt having been made thus to create uniform moisture conditions for bacterial growth.

Varying quantities of soil, namely, twenty-five, fifty, seventy-five, one hundred and two hundred gms. were used. The several portions of soil were kept in glass tumblers covered with Petri plates. Ammonia determinations were made at the end of three days in the case of the peptone; at the end of six days in the case of the dried blood; and at the end of seven days in the case of the cottonseed meal. The arrangement of the entire series was as follows:

Soil No.						
1, 2	25	gms.	soil +	0.5	gm.	peptone.
3, 4	50	"	"	"	"	
5, 6	75	"	"	"	"	
7, 8	100	"	"	"	"	
9, 10	200	"	"	"	"	
11, 12	25	gms.	soil +	1.0	gm.	peptone.
13, 14	50	"	"	"	"	
15, 16	75	"	"	"	"	
17, 18	100	"	"	"	"	
19, 20	200	"	"	"	"	
21, 22	25	gms.	soil +	2.0	gms.	peptone.
23, 24	50	"	"	"	"	
25, 26	75	"	"	"	"	
27, 28	100	"	"	"	"	
29, 30	200	"	"	"	"	
31, 32	25	gms.	soil +	5	gms.	dried blood.
33, 34	50	"	"	"	"	"
35, 36	75	"	"	"	"	"
37, 38	100	"	"	"	"	"
39, 40	200	"	"	"	"	"
41, 42	25	gms.	soil +	10	gms.	dried blood.
43, 44	50	"	"	"	"	"
45, 46	75	"	"	"	"	"
47, 48	100	"	"	"	"	"
49, 50	200	"	"	"	"	"

Soil No.						
51, 52	25	gms.	soil +	20	gms.	dried blood.
53, 54	50	"	"	"	"	"
55, 56	75	"	"	"	"	"
57, 58	100	"	"	"	"	"
59, 60	200	"	"	"	"	"
61, 62	25	gms.	soil +	5	gms.	cottonseed meal.
63, 64	50	"	"	"	"	"
65, 66	75	"	"	"	"	"
67, 68	100	"	"	"	"	"
69, 70	200	"	"	"	"	"
71, 72	25	gms.	soil +	10	gms.	cottonseed meal.
73, 74	50	"	"	"	"	"
75, 76	75	"	"	"	"	"
77, 78	100	"	"	"	"	"
79, 80	200	"	"	"	"	"
81, 82	25	gms.	soil +	20	gms.	cottonseed meal.
83, 84	50	"	"	"	"	"
85, 86	75	"	"	"	"	"
87, 88	100	"	"	"	"	"
89, 90	200	"	"	"	"	"

It will be noted that the arrangement of the present experiment is such as to bring out among other things the influence of varying quantities of soil, in other words, of varying quantities of mineral matter and of varying numbers of bacteria, on the rate of decay of nitrogenous materials. The yields of ammonia nitrogen were as follows:

Soil No.	*Feb. 3–6.*	*Average.*	*Soil No.*	*Feb. 3–6.*	*Average.*
1	40.97 mg.		16	85.12 mg.	85.75 mg.
2	40.81 "	40.89 mg.	17	84.96 "	
3	45.27 "		18	81.29 "	83.12 "
4	44.95 "	45.11 "	19	95.32 "	
5	47.02 "		20	72.69 "	84.00 "
6	46.07 "	46.54 "	21	72.21 "	
7	46.23 "		22	64.88 "	68.54 "
8	46.54 "	46.38 "	23	125.93 "	
9	45.11 "		24	119.39 "	122.66 "
10	*116.20 "	45.11 "	25	140.91 "	
11	62.00 "		26	141.23 "	141.07 "
12	64.08 "	63.04 "	27	149.84 "	
13	84.32 "		28	145.85 "	147.84 "
14	79.70 "	82.01 "	29	150.79 "	
15	86.39 "		30	148.88 "	149.83 "

* Not included in the average.

As we examine the data tabulated above we note again the increasing production of ammonia in the larger applications of peptone. For instance, with seventy-five gms. of soil, 0.5 peptone yielded 45.11 mgs. of ammonia nitrogen; 1.0 gm. peptone yielded 82.01 mgs. ammonia nitrogen, and 2.0 gms. peptone yielded 122.66 mgs. ammonia nitrogen. The peptone contains, apparently, a portion of its nitrogen in a condition readily affected by the bacterial activities; this portion must be very considerable since one gm. of peptone, containing about 160 mgs. of nitrogen, yielded more than eighty mgs. of ammonia nitrogen in three days.

The most instructive point, however, brought out by the above data is the almost constant amount of ammonia produced in fifty, seventy-five, one hundred and two hundred gms. of soil from any given quantity of peptone. It is only in the case of the two gms. of peptone that the production of ammonia was markedly smaller in the fifty gms. of soil. On the other hand, the production of ammonia was decidedly smaller in the twenty-five gms. of soil; moreover, the differences between the twenty-five and fifty gms. portions increased as the amount of peptone grew larger.

As to the smaller yields of ammonia in the twenty-five gms. portions of soil, they may have been due to the more rapid drying out of the material, to the smaller supply of mineral nutrients for the bacteria, or, perhaps, to the smaller number of micro-organisms present. The rather uniform yields in the other soil portions could be accounted for by the assumption that the optimum conditions as to mineral food and numbers of bacteria were already attained in the fifty gm. portions and the additional quantities of soil could not intensify still further the rate of transformation; indeed the intensity of the transformation of the peptone nitrogen made it appear, almost, as if we had to deal here with enzyme action. As will be shown presently, ammonia formation from dried blood bore a different relation to the amounts of soil employed.

Soil No.	*Feb. 3–9.*	*Average.*	*Soil No.*	*Feb. 3–9.*	*Average.*
31	11.80 mg.		46	41.92 mg.	40.80 mg.
32	7.49 "	9.64 mg.	47	54.20 "	
33	20.24 "		48	52.38 "	53.29 "
34	16.74 "	18.49 "	49	88.63 "	
35	30.92 "		50	89.10 "	88.86 "
36	33.95 "	32.43 "	51	36.34 "	
37	37.62 "		52	35.54 "	35.94 "
38	38.26 "	37.94 "	53	41.28 "	
39	54.20 "		54	40.65 "	40.96 "
40	54.04 "	54.12 "	55	50.05 "	
41	20.08 "		56	54.20 "	52.12 "
42	20.56 "	20.32 "	57	73.32 "	
43	28.37 "		58	65.35 "	69.33 "
44	27.74 "	28.05 "	59	137.56 "	
45	39.69 "		60	121.14 "	129.30 "

As we glance over the average yields of ammonia from the dried blood, we note that the increasing quantities of soil were followed by an increasing production of ammonia from the same quantity of dried blood. We note also that larger quantities of dried blood yielded larger quantities of ammonia; hence, we may conclude that ammonia formation from dried blood was affected by the amount of soil as well as the amount of dried blood used; whereas in the case of peptone the amount of soil, within certain limits, did not affect the yields of ammonia nitrogen. Now, peptone is a mixture of cleavage products derived from protein bodies, while dried blood consists of protein substances. But when we come to cottonseed meal we have a substance that contains not only protein bodies, but also carbohydrates which modify the decomposition of the nitrogenous compounds by bacteria; hence, the yields of ammonia from cottonseed meal show relations somewhat different from those already observed in the case of peptone and dried blood. This is shown in the following table:

Soil No.	*Feb. 3–10.*	*Average.*	*Soil No.*	*Feb. 3–10.*	*Average.*
61	75.24 mg.		76	185.22 mg.	196.70 mg.
62	82.25 "	78.74 mg.	77	185.38 "	
63	108.23 "		78	192.40 "	188.89 "
64	106.00 "	107.11 "	79	148.24 "	
65	101.06 "		80	144.58 "	146.41 "
66	104.25 "	102.65 "	81	82.73 "	
67	91.50 "		82	72.21 "	77.47 "
68	76.83 "	84.16 "	83	191.76 "	
69	64.24 "		84	227.46 "	209.61 "
70	67.43 "	65.83 "	85	286.92 "	
71	81.13 "		86	277.99 "	282.45 "
72	103.61 "	92.37 "	87	363.75 "	
73	166.73 "		88	397.86 "	380.80 "
74	181.24 "	173.98 "	89	386.86 "	
75	208.18 "		90	354.82 "	370.84 "

It is evident from the data just given that the larger quantities of cottonseed meal yielded larger amounts of ammonia; this we may readily see by comparing soil portions 64, 74, 84, 65, 75 and 85, &c. When, however, we attempt to determine the influence of the varying amounts of soil we fail to secure definite results; for instance, with ten gms. of cottonseed meal twenty-five gms. of soil produced, on the average, 78.14 mgs. of ammonia nitrogen, and fifty gms. of soil, 107.11 mgs. of ammonia nitrogen. After that, however, the yields of ammonia decreased rather than increased. Similarly in the case of ten gms. of cottonseed meal there was an increase up to seventy-five gms. of soil, and then a gradual decrease, while in the case of twenty gms. of cottonseed meal there was an increase up to 100 gms. of soil and then a slight decrease.

We are unable to account definitely for the discrepancies just noted, but may suggest some factors that probably contributed to the results in question; in the first place, the soils to which cottonseed meal was added developed a heavy growth of mucors, penicillia, &c. These molds probably produced some ammonia and used up more than they produced. In the second place, the larger amounts of soil, by diluting the cottonseed meal, and by furnishing larger quantities of mineral salts, modified the species relationships so as to decrease the accumulation though not necessarily the formation of ammonia in the soil. In the third place, the carbohydrates supplied by the cottonseed meal affected both numbers and species of decay bacteria to an unequal extent in the varying quantities of soil.

Everything considered, therefore, the results secured in the present series are suggestive in furnishing us with new points of view on decay and ammonia formation in soils. They show us that the production of available nitrogen compounds is affected not only by the amount of nitrogenous material present or applied, but also by the depth of soil; that is, the absolute quantity of soil in which a given quantity of nitrogenous fertilizer is distributed.

AMMONIA PRODUCTION FROM PEPTONE, DRIED BLOOD AND COTTONSEED MEAL IN VARYING QUANTITIES OF SOIL, BUT WITH A CONSTANT BACTERIAL CONTENT.

SERIES E.

The factors that affected ammonia formation in the preceding experiments were assumed to include numbers of bacteria and the supply of mineral salts. Evidently fifty gms. of soil would supply not only much greater numbers of bacteria, but also more soluble sulphates, phosphates, chlorides, &c., than would be supplied in twenty-five gms. of the same soil. Hence, it seemed advisable to arrange the present experiment so as to make the bacterial factor constant and only the supply of mineral salts variable. Accordingly, fifty, one hundred and two hundred gm. portions of soil were sterilized in the autoclave and mixed with varying amounts of sterile peptone, dried blood and cottonseed meal. Each soil portion was then inoculated with soil infusion bacteriologically equivalent to ten gms. of soil. There were thus present, at the beginning of the experiment, the same number of bacteria in each soil portion.

The soils were kept in covered tumblers at room temperature. Ammonia determinations were made at the end of four days in the case of the peptone and at the end of seven days in the case of the dried blood and cottonseed meal. Additional amounts of sterile water were supplied to the soil portions that had received the larger quantities of dried blood and cottonseed meal, so as to create the same relative conditions as to moisture in all of the soils. The arrangement of this series was as follows:

Soil No.	*Addition.*	*Amount of Soil.*
1, 2	0.5 gm. peptone.	50.0 gms.
3, 4	1.0 " "	
5, 6	2.0 gms. "	
7, 8	0.5 gm. "	100.0 gms.
9, 10	1.0 " "	
11, 12	2.0 gms. "	
13, 14	0.5 gm. "	200.0 gms.
15, 16	1.0 " "	
17, 18	2.0 gms. "	

Soil No.	*Addition.*	*Amount of Soil.*
19, 20	5.0 gms. dried blood.	50.0 gms.
21, 22	10.0 " " "	
23, 24	20.0 " " "	
25, 26	5.0 " " "	100.0 gms.
27, 28	10.0 " " "	
29, 30	20.0 " " "	
31, 32	5.0 " " "	200.0 gms.
33, 34	10.0 " " "	
35, 36	20.0 " " "	

Soil No.	*Addition.*	*Amount of Soil.*
37, 38	5.0 gms. cottonseed meal.	50.0 gms.
39, 40	10.0 " " "	
41, 42	20.0 " " "	
43, 44	5.0 " " "	100 gms.
45, 46	10.0 " " "	
47, 48	20.0 " " "	
49, 50	5.0 " " "	200 gms.
51, 52	10.0 " " "	
53, 54	20.0 " " "	

The amounts of ammonia nitrogen found in the several soil portions were as follows:

Soil No.		*Soil No.*		*Average.*	*Soil No.*	
1	52.76 mg.	2	53.72 mg.	53.74 mg.	19	55.63 mg.
3	94.36 "	4	91.50 "	92.93 "	21	25.98 "
5	124.01 "	6	124.97 "	124.49 "	23	48.62 "
7	52.60 "	8	54.67 "	53.63 "	25	105.84 "
9	102.65 "	10	106.16 "	104.40 "	27	113.49 "
11	190.96 "	12	191.92 "	191.44 "	29	51.49 "
13	54.51 "	14	53.72 "	54.11 "	31	84.32 "
15	98.83 "	16	101.06 "	99.94 "	33	154.14 "
17	191.28 "	18	193.99 "	192.63 "	35	177.73 "

Soil No.		*Average.*	*Soil No.*		*Soil No.*		*Average.*
20	48.62 mg.	52.12	37	119.39 mg.	38	119.07 mg.	119.23 mg.
22	26.62 "	26.30	39	117.96 "	40	117.00 "	117.48 "
24	47.34 "	47.98	41	190.16 "	42	102.02 "	146.09 "
26	76.99 "	91.41	43	103.29 "	44	109.51 "	106.40 "
28	111.58 "	112.53	45	192.71 "	46	200.84 "	196.77 "
30	87.51 "	69.50	47	262.21 "	48	224.59 "	243.40 "
32	86.39 "	85.35	49	84.00 "	50	76.99 "	80.49 "
34	151.43 "	158.72	51	137.08 "	52	140.11 "	138.59 "
36	172.31 "	175.02	53	208.34 "	54	201.96 "	205.15 "

With the same number of bacteria supplied in each case, we find increasing yields from the larger applications of peptone. Thus the average yields of ammonia nitrogen in soil portions 1 and 2, 3 and 4, 5 and 6 were 53.74 mg., 92.93 mg., and 124.49 mg., respectively; similar results were secured with 100 gms. and 200 gms. of soil.

As to the influence of varying quantities of soil with 0.5 gm. of peptone, it is scarcely apparent in the yield of ammonia nitrogen. The average production of ammonia nitrogen was 53.74 mg.

in fifty gms. of soil; 53.63 mg. for 100 gms. of soil, and 54.11 mg. for 200 gms. of soil. The corresponding yields from 1.0 of peptone do not show any very marked effect from the variation in the amount of soil used. On the other hand, with 2.0 gms. of peptone the yields of ammonia in the fifty gm. portions of soil were smaller than those in the 100 gm. and 200 gm. portions.

It seems, thus, that with an initially constant number of bacteria the production of ammonia from small quantities of peptone was not affected by the amount of soil employed within the given limits. When, however, the peptone was present in amounts greater than one gm. the production of ammonia was influenced also by the quantity of soil used; it may be concluded, therefore, that either the supply of mineral salts in the soil or the modified conditions of aeration are responsible for the results just noted.

When we examine the corresponding results secured with dried blood we find relations that are different. In the first place, the increased application of dried blood was not always followed by a corresponding increase in the amount of ammonia produced. For instance, with fifty gms. of soil the average yield of ammonia from five, ten and twenty gms. of dried blood was 52.12 mg., 26.30 mg. and 47.98 mg., respectively. Irregular returns were secured also from the 100 gm. portions of soil. On the other hand, the larger amounts of dried blood in the 200 gm. portions of soil yielded correspondingly larger amounts of ammonia. In the case of the cottonseed meal the influence of the larger applications is, as a rule, reflected in the yields of ammonia. Even here, however, there is more or less irregularity in the yields and in the proportionate increase. It would seem as if much larger numbers of bacteria are required at the beginning for the cottonseed meal and dried blood than are required for the peptone in order to establish uniform bacteriological conditions for ammonification. At present it is difficult to determine in how far the more uniform transformation of the peptone was due to its chemical composition, and in how far to its solubility and, therefore, better distribution in the soil.

Inquiring into the influence of the soil quantity employed on the ammonification of the dried blood and cottonseed meal, we find that in the case of the former this influence was undoubtedly felt. The total yield of ammonia nitrogen was 126.40 mg. for the fifty gm. portions of soil; 273.44 mg. for the 100 gm. portions, and

449.09 mg. for the 200 gm. portions. Nevertheless, the results were not as consistent as they were in series D, and we must conclude that the larger yields of ammonia from dried blood in the larger quantities of soil were influenced directly both by the numbers of bacteria supplied and the chemical and physical conditions created by the soil itself.

In the case of the cottonseed meal the total yields of ammonia nitrogen in the fifty gms., 100 gms. and 200 gms. of soil, were 382.80 mg., 546.57 mg. and 424.23 mg. Turning back to series D we find that also there the larger quantities of soil yielded, in a number of instances, smaller quantities of ammonia. Now, since the initial number of bacteria was constant in the present series it would appear that the soil itself had, in some way, something to do with the results noted.

AMMONIA PRODUCTION FROM PEPTONE AND DRIED BLOOD IN CONSTANT QUANTITIES OF SOIL WITH A VARYING BACTERIAL CONTENT.

SERIES F.

For the sake of securing additional information as to the bacteriological factor in the ammonification of peptone, dried blood and cottonseed meal, 100 gm. portions of sterilized fertile soil were inoculated with varying quantities of soil infusion, equivalent to 2.5, five and ten gms., respectively, of fresh soil. The treatment of the several soil portions may be best seen from the following plan:

Soil No.	*Soil Infusion Equivalent to*	
1, 2	2.5 gms. of soil.	0.5 gms. peptone.
3, 4	5.0 " " "	
5, 6	10.0 " " "	
7, 8	2.5 " " "	2.0 " "
9, 10	5.0 " " "	
11, 12	10.0 " " "	

Soil No.	*Soil Infusion Equivalent to*	
13, 14	2.5 gms. of soil.	5.0 gms. dried blood.
15, 16	5.0 " " "	
17, 18	10.0 " " "	
19, 20	2.5 " " "	20.0 " " "
21, 22	5.0 " " "	
23, 24	10.0 " " "	

Soil No.	*Soil Infusion Equivalent to*	
25, 26	2.5 gms. of soil.	5.0 gms. of cottonseed meal.
27, 28	5.0 " " "	
29, 30	10.0 " " "	
31, 32	2.5 " " "	20.0 " " " "
33, 34	5.0 " " "	
35, 36	10.0 " " "	

Additional quantities of sterile water were supplied wherever necessary in order to establish uniform moisture conditions in all of the soil portions. As in the preceding series the soils were kept in covered tumblers. Ammonia determinations were made in soil portions 1 to 12 in five days, and in soil portions 13 to 36 in seven days. The amounts of ammonia nitrogen found in the several soils are given in the following tabulation:

Soil No.	*Ammonia N.*	*Average.*	*Soil No.*	*Ammonia N.*	*Average.*
1	55.95 mg.		19	43.36 mg.	
2	57.54 "	56.74 mg.	20	45.43 "	44.39 mg.
3	58.66 "		21	44.47 "	
4	57.70 "	58.18 "	22	43.52 "	43.99 "
5	57.38 "		23	43.04 "	
6	58.50 "	57.94 "	24	43.99 "	43.51 "
7	181.72 "		25	32.68 "	
8	198.77 "	190.24 "	26	24.71 "	28.69 "
9	199.41 "		27	34.91 "	
10	182.83 "	191.12 "	28	25.82 "	30.36 "
11	203.08 "		29	100.10 "	
12	183.31 "	193.19 "	30	73.96 "	87.03 "
13	18.49 "		31	201.00 "	
14	14.51 "	16.50 "	32	230.33 "	215.66 "
15	*46.34 "		33	289.47 "	
16	16.26 "	16.26 "	34	222.58 "	256.02 "
17	31.72 "		35	273.21 "	
18	24.71 "	28.21 "	36	275.44 "	274.32 "

* Not included in the average.

In the preceding series the number of bacteria supplied was constant and the amount of sterile soil variable. In the present series the number of bacteria supplied was variable and the amount of soil constant. Now, the smallest number of bacteria supplied here was equivalent to that present in 2.5 gms. of fertile soil, and the largest number was four times as great. Within these limits the ammonification of peptone in 100 gms. of sterile soil was not markedly influenced by the variation in the quantity of inoculating material. It will be observed that the ammonia formed from 0.5 gms. of peptone was substantially the same throughout and the same may be said of the ammonia formed from the two gms. quantities of peptone.

In the case of the dried blood the results were not always as definite as they were in the case of the peptone. There was, nevertheless, a tendency towards uniformity also here in so far as the production of ammonia from any given amount of material was concerned. We find, at any rate, that five gms. of dried blood yielded about sixteen mgs. of ammonia nitrogen when the inoculating material was equivalent to 2.5 or 5.0 gms. of fresh soil. When the inoculating material was equivalent to 10.0 gms. of soil the amount of ammonia nitrogen formed was markedly larger. With 20.0 gms. of dried blood, however, the ammonia formed was rather constant in quantity; similar relations may be observed also in the case of the cottonseed meal, although there was here a tendency towards more extensive ammonia formation as the amount of inoculating material was increased. It was thus indicated by the facts in the case that the numbers of bacteria adequate for the ammonification of fairly large quantities of readily decomposable material, or of small quantities of more inert nitrogenous material, are insufficient for larger amounts of inert material. Further evidence in this direction is furnished by the following series.

SERIES G.

One hundred gram quantities of soil were placed in stoppered Jena flasks and supplied either with one gm. sterile peptone or five gms. sterile dried blood. Sterile water was added up to optimum amounts and the different soil portions were then inoculated with quantities of soil infusion corresponding to varying amounts of soil. The treatment of the several soil portions in this experiment is shown in the following table:

No. of Soil Portions.	*Inoculation Equivalent.*	
1, 2	.....	
3, 4	0.1 gm. of soil.	1 gm. peptone per 100 gms. soil.
5, 6	0.5 " "	
7, 8	1.0 " "	
9, 10	2.0 gms. "	
11, 12	.....	
13, 14	0.1 gm. of soil.	5 gms. dried blood per 100 gms. soil.
15, 16	0.5 " "	
17, 18	1.0 " "	
19, 20	2.0 gms. "	

The several soil portions were kept in the incubator at 28° C. Ammonia determinations were made in soils 1 to 10 at the end of three days, and in soils 11 to 20 at the end of six days. The amounts of ammonia nitrogen found were as follows:

No.	*Ammonia N.*	*No.*	*Ammonia N.*	*Average.*
1	38.43* mg.	2	4.25 mg.	4.25 mg.
3	85.52 "	4	78.43 "	81.97 "
5	91.03 "	6	88.83 "	89.93 "
7	89.46 "	8	91.66 "	90.56 "
9	91.51 "	10	86.78 "	89.14 "
11	3.78 "	12	2.05 "	2.91 "
13	26.46 "	14	31.34 "	28.90 "
15	39.37 "	16	32.13 "	35.75 "
17	22.52 "	18	18.11 "	20.31 "
19	45.52 "	20	26.62 "	36.07 "

* Not included in the average.

As is indicated by the data in the above table a comparatively small number of bacteria is sufficient for the ammonification of peptone. The amount of ammonia nitrogen found in the uninoculated soil portion 1 may be taken to represent that formed by purely chemical means during the process of sterilization. It was 4.25 mg. The duplicate soil portion 1 had evidently become contaminated and was not included, therefore, in the average. Soil portions 3 and 4 contained, on the average, 81.97 mg. ammonia nitrogen, most of it produced by the bacteria in 0.1 gm. of soil. Soil portions 5 to 10, which received gradually increasing numbers of bacteria in the inoculating material, contained but little more ammonia than soil portions 3 and 4; indeed, soil portions 9 and 10 each inoculated with a bacterial equivalent of 2 gms. of soil, contained essentially the same amount of ammonia as soil portions 5 and 6, each of which was inoculated with a bacterial equivalent of only 0.5 gm. of soil.

But when we compare the yields of ammonia from the dried blood in soil portions 11 to 20 we fail to find similar uniformity. The more inert character of the dried blood prevented rapid cleavage along the same lines, and differences in numbers and species were evidently of more influence here than in the soil portions 3 to 10, containing peptone. We note, however, not only a greater variation in the duplicates, but also a tendency towards the production of more ammonia nitrogen in some of the soil portions as the amount of inoculating material was increased. Thus in soil portions 13 and 14 the average production of ammonia was 28.90 mg., while in soil portions 15 and 16 it was 35.75 mg. and in soil portions 19 and 20, 36.07 mg.

THE CHEMICAL AND BACTERIOLOGICAL FACTORS IN THE AMMONIFICATION AND NITRIFICATION OF NITROGENOUS MATERIALS IN THE SOIL.

SERIES H.

In experiments conducted by one of us in 1905 and 1906 an attempt was made to differentiate the chemical and bacteriological factors in the ammonification of peptone*. The results secured with 1 per cent. solutions of this material led to the conclusion that both the chemical and bacteriological factors played a part in the production of ammonia from peptone or similar materials.

In the present experiments dried blood was used instead of peptone, with the further difference that the substance to be ammonified was mixed in each case with 100 gms. of soil instead of being dissolved or suspended in 100 c.c. of water.

A number of 100 gm. portions of greenhouse soils, 1, 2, 3, 5 and 7 employed in the experiments already recorded in this paper were arranged in three groups as follows:

a—100 gm. quantities of soil, mixed with five gm. of dried blood, and twenty-five c.c. of sterile water were placed in covered beakers and kept in the incubator.

b—100 gm. quantities of the same soils were mixed with five gms. of dried blood, placed in stoppered erlenmeyers, sterilized and inoculated, on cooling, with twenty c.c. infusion prepared by shaking 100 gms. of fertile soil with 200 c.c. of sterile water. The twenty c.c. of infusion used in each case were therefore equivalent to ten gms. of soil. Aside from the infusion, each soil portion received also a further addition of five c.c. of sterile water. The flasks and soil were kept in the incubator like the soil portions of group *a*.

c—100 gm. portions of the fertile soil employed for the preparation of infusions for group *b* were mixed with five gms. dried blood, sterilized in erlenmeyer flasks and were inoculated on cooling with infusions from the several soils of group *a*. The twenty c.c. of infusion equivalent in each case to ten gms. of soil were supplemented by five c.c. of sterile water.

* Report of the Soil Chemist and Bacteriologist, N. J. Sta. Rep., 1906, p. 119.

The treatment of the soils in each group and the amounts of ammonia produced in six days are recorded in the following table:

GROUP *a*.

Soil No.	*Ammonia N.*	*Average.*
1	6.01 mg.	
1	7.95 "	6.98 mg.
2	6.89 "	
2	7.95 "	7.42 "
3	8.13 "	
3	8.30 "	8.21 "
5	7.60 "	
5	6.53 "	7.06 "
7	7.42 "	
7	7.95 "	7.68 "

GROUP *b*.

Soil No.	*Ammonia N.*	*Average.*
1	89.94 mg.	
1	81.28 "	85.61 mg.
2	65.38 "	
2	72.27 "	68.82 "
3	71.21 "	
3	59.55 "	65.38 "
5	69.09 "	
5	92.23 "	80.66 "
7	78.81 "	
7	88.70 "	80.75 "

GROUP *c*.

Infusion of Soil No.	*Ammonia N.*	*Average.*
1	72.80 mg.	
1	69.80 "	71.30 mg.
2	74.57 "	
2	70.86 "	72.71 "
3	70.15 "	
3	58.66 "	64.40 "
5	74.16 "	
5	56.72 "	65.44 "
7	47.53 "	
7	61.31 "	54.42 "

We find from the data just given that the production of ammonia in soils 1, 2, 3, 5 and 7 was comparatively slight (group *a*), and that the amounts thus produced were fairly uniform. On the other hand, when the same soils were first sterilized in the autoclave and then inoculated with equal amounts of infusion from a fertile soil the amounts of ammonia produced were very considerable (group *b*). Similarly, when 100 gm. quantities of the

fertile soil were previously sterilized and then inoculated with equivalent amounts of infusion from soils 1, 2, 3, 5 and 7 (group *c*) the production of ammonia was nearly, though not quite, as great as it was in group *b*. It appears, therefore, that in the unsterilized soils 1, 2, 3, 5 and 7 ammonification was comparatively feeble, possibly because of the previous accumulation of bacterial products injurious to the micro-organisms. On the other hand, the same soils when previously sterilized were found capable of supporting a more intense ammonification in a fertile soil (group *b*); while the bacteria from these soils were also capable of producing a much larger quantity of ammonia when transferred to sterile portions of a fertile soil (group *c*). As to the influence of previous treatment of soils 1, 2, 3, 5 and 7 the lack of satisfactory agreement in the duplicates renders definite deductions impracticable.

SERIES I.

Soils 1, 2, 3, 5 and 7, used in the preceding series, were employed also here. In the present series, however, an attempt was made to study the chemical and bacteriological factors of *nitrification* instead of *ammonification*. The arrangement of this series was as follows:

Group *a*—100 gm. quantities of soils 1, 2, 3, 5 and 7 were mixed either with 100 mgs. ammonium sulphate or 200 mgs. of dried blood, placed in covered tumblers and maintained at optimum moisture conditions for four weeks. The several soil portions were numbered as follows:

Soil No.	*Soil Portion.*	*Addition.*
1	20	Blank.
	21	100 mgs. $(NH_4)_2 SO_4$.
	22	200 " dried blood.
2	23	Blank.
	24	100 mgs. $(NH_4)_2 SO_4$.
	25	200 " dried blood.
3	26	Blank.
	27	100 mgs. $(NH_4)_2 SO_4$.
	28	200 " dried blood.
5	29	Blank.
	30	100 mgs. $(NH_4)_2 SO_4$.
	31	200 " dried blood.
7	32	Blank.
	33	100 mgs. $(NH_4)_2 SO_4$.
	34	200 " dried blood.

Group *b*—100 gm. quantities of soils 1, 2, 3, 5 and 7 were placed in stoppered erlenmyer flasks, sterilized in the autoclave and mixed either with 100 mgs. of sterile $(NH_4)_2\ SO_4$ or 200 mgs. of sterile dried blood. They were each inoculated on cooling with twenty c.c. of infusion corresponding to ten gms. of the fertile soil employed in the preceding series. The several soil portions in this group were numbered as follows:

Soil No.	*Soil Portion.*	*Addition.*
1	35	100 mgs. $(NH_4)_2\ SO_4$.
	36	200 " dried blood.
2	37	100 mgs. $(NH_4)_2\ SO_4$.
	38	200 " dried blood.
3	39	100 mgs. $(NH_4)_2\ SO_4$.
	40	200 " dried blood.
5	41	100 mgs. $(NH_4)_2\ SO_4$.
	42	200 " dried blood.
7	43	100 mgs. $(NH_4)_2\ SO_4$.
	44	200 " dried blood.

These soil portions, like those of Group *a*, were maintained at optimum moisture conditions by periodical additions of sterile water, and were kept in the incubator for four weeks.

Group *c*—100 gm. quantities of fertile soil were placed in stoppered erlenmyer flasks, sterilized, supplied either with 100 mgs. of ammonium sulphate or 200 mgs. of dried blood, and inoculated in each case with twenty c.c. of infusion corresponding to ten gms. of soils 1, 2, 3, 5 and 7. Optimum moisture conditions were maintained by periodical additions of sterile water. Nitrate and nitrite determinations were made as in Groups *a* and *b* at the end of four weeks. The several soil portions in this group were numbered as follows:

Soil Infusion No.	*Soil Portion.*	*Addition.*
1	45	100 mgs. $(NH_4)_2\ SO_4$.
	46	200 " dried blood.
2	47	100 mgs. $(NH_4)_2\ SO_4$.
	48	200 " dried blood.
3	49	100 mgs. $(NH_4)_2\ SO_4$.
	50	200 " dried blood.
5	51	100 mgs. $(NH_4)_2\ SO_4$.
	52	200 " dried blood.
7	53	100 mgs. $(NH_4)_2\ SO_4$.
	54	200 " dried blood.

The amounts of nitrate and nitrite nitrogen found in the several soil portions were as follows:

GROUP *a*.

Soil. No.	*Soil Portion.*	*Nitrate N.*	*Nitrite N.*
1	20	2.000 mg.	0.0980 mg.
	21	0.832 "	0.0868 "
	22	0.062 "	0.0608 "
2	23	0.125 "	0.0522 "
	24	4.000 "	0.0608 "
	25	6.664 "	0.1104 "
3	26	0.050 "	0.0680 "
	27	2.150 "	0.1012 "
	28	0.062 "	0.0676 "
5	29	5.000 "	0.0948 "
	30	1.955 "	0.0605 "
	31	3.332 "	0.0672 "
7	32	3.552 "	0.0760 "
	33	8.000 "	0.0608 "
	34	8.552 "	0.0712 "

GROUP *b*.

Soil. No.	*Soil Portion.*	*Nitrate N.*	*Nitrite N.*
1	35	10.000 mg.	0.0932 mg.
	36	0.054 "	0.0808 "
2	37	0.060 "	0.0582 "
	38	0.153 "	0.1736 "
3	39	0.054 "	0.0600 "
	40	0.832 "	0.1620 "
5	41	0.248 "	0.0812 "
	42	1.428 "	0.1520 "
7	43	0.200 "	0.0640 "
	44	0.160 "	0.2208 "

GROUP *c*.

Soil. No.	*Soil Portion.*	*Nitrate N.*	*Nitrite N.*
1	45	2.000 mg.	0.1104 mg.
	46	0.143 "	0.0592 "
2	47	0.600 "	0.1216 "
	48	2.000 "	0.1620 "
3	49	0.520 "	0.1013 "
	50	1.000 "	0.1518 "
5	51	0.240 "	0.1008 "
	52	2.100 "	0.0868 "
7	53	0.488 "	0.1102 "
	54	0.312 "	0.1739 "

The above data are difficult to interpret. Soils 1 and 2 received the same treatment in the greenhouse and should have given the same results. At the same time they show wide differences, not only in the soil portions that had received no additions of nitrogenous material (portions 20 and 23), but also in those that received additions of ammonium sulphate on the one hand (portions 21 and 24) and of dried blood on the other (portions 22 and 25). We find, further, that in soil 3, which had received the smaller addition of dextrose, there were produced 2.150 mgs. nitrate nitrogen where ammonium sulphate was used (portion 27), and only 0.062 mgs. where dried blood was used. On the other hand, in soil 5, the production of nitrate from the dried blood was greater than that from the ammonium sulphate, and in either case it was less than that produced in the same soil when no nitrogenous material was added (portion 29). In soil 7, which received an application of sodium citrate, the production of nitrate was more extensive than in any of the other soils. On the whole, the dried blood yielded more nitrate nitrogen than the ammonium sulphate in this group.

In Group *b* the production of nitrate was comparatively slight, if we except portion 35. It seems that the sterilization of the soil had unfavorably influenced, for the time being, the activities of the nitrifying bacteria subsequently introduced in the soil infusion. In Group *c*, where sterile portions of the fertile soil were inoculated with equivalent amounts of infusion from soils 1, 2, 3, 5 and 7, the production of nitrate was more pronounced. In no case, however, did it represent more than a small proportion of the nitrogen contained in the materials added. It may be pointed out, also, that the determinations of nitrite nitrogen brought out nothing distinctive. In some instances a high nitrite content corresponded to a high nitrate content, yet the amounts of nitrite nitrogen were not proportional to the amounts of nitrate nitrogen. For the rest it would not be safe to regard the data at hand as throwing much light on the relations of the chemical and bacteriological factors of nitrification under the conditions of the present experiment.

SERIES J.

Five soils marked A, B, C, D and E, respectively, were used in this series for the differentiation of the chemical and bacteriological factors of ammonification. Soil A came from Jerseyville; soil B from Farmingdale; soil C from Haddonfield; soil D from Colts Neck, and soil E from New Brunswick. The several soil portions were arranged in three groups, *a*, *b*, and *c*, as in preceding series. In each case 100 gms. of soil were mixed with five gms. of dried blood and maintained at optimum moisture conditions for six days. In Group *a* fresh soil was employed and the 100 gm. portions were kept in covered beakers. In Group *b* 100 gm. portions of the different soils were mixed with the dried blood placed in stoppered erlenmyer flasks, sterilized and inoculated with equal amounts of infusion from a fertile soil. In Group *c* 100 gm. portions of the fertile soil were treated and sterilized in the same manner, and then inoculated with equivalent amounts of infusion from soils A to E. Group *a* was thus to represent the influence of both the chemical and bacteriological factors of soils A to E; Group *b* was to represent the chemical factors of these soils, and Group *c* the bacteriological factors of these soils. The following amounts of ammonia nitrogen were found in the several soil portions:

GROUP *a*.

Soil No.	*Soil Portion No.*	*Ammonia N.*	*Average.*
A	61	37.46 mg.	
	62	33.93 "	35.69 mg.
B	63	51.24 "	
	64	61.31 "	56.27 "
C	65	45.76 "	
	66	41.70 "	43.73 "
D	67	27.21 "	
	68	36.05 "	31.63 "
E	69	12.72 "	
	70	21.38 "	17.05 "

GROUP *b*.

Soil No.	*Soil Portion No.*	*Ammonia N.*	*Average.*
A	71	36.22 mg.	
	72	27.03 "	31.62 mg.
B	73	71.03 "	
	74	65.56 "	68.29 "
C	75	108.85 "	
	76	125.28 "	117.06 "
D	77	106.72 "	
	78	107.61 "	107.16 "
E	79	158.68 "	
	80	154.26 "	156.47 "

GROUP *c*.

Soil No.	*Soil Portion No.*	*Ammonia N.*	*Average.*
A	81	45.23 mg.	
	82	41.35 "	43.29 mg.
B	83	51.60 "	
	84	55.48 "	53.54 "
C	85	52.12 "	
	86	57.07 "	54.59 "
D	87	50.36 "	
	88	46.29 "	48.32 "
E	89	48.59 "	
	90	53.19 "	50.89 "

The five soils in this experiment showed considerable differences as to their ability to produce ammonia out of dried blood. For instance, in soil B the average amount of ammonia nitrogen produced was 56.27 mgs., while in soil E it was only 17.05 mgs. But when these soils were previously sterilized and then inoculated with equal quantities of the same soil infusion (Group *b*) their ability to support ammonification was variable, and the differences did not correspond to those in Group *a*. We see, thus, that soil E, which, unsterilized, produced 17.05 mgs. of ammonia nitrogen led to the production, after sterilization of 156.47 mgs. On the other hand, soil B, which produced the largest amount of ammonia nitrogen in Group *a*, produced next to the smallest amount in Group *b*.

Turning now to Group *c* we find that with the chemical factor constant, and the bacteriological factor variable the differences in ammonia production were not very great. Soils C and B gave the highest yield, while soil A gave the lowest yield. It seems, therefore, that the chemical factor in ammonification causes a much

wider range in ammonia production than the bacteriological factor. It appears, also, that in the unsterilized soil there may be an accumulation of organisms or substances capable of retarding ammonification, the former, as well as a portion of the latter may be destroyed by sterilization. This view finds support in the fact that there is periodicity not only in nitrification, but also in ammonification. As to the causes of this periodicity and the chemical and biological factors involved our knowledge is very scant. Methods like those employed in the present experiments as well as in the modifications of these methods, that will be introduced in the course of time, should supply information relating to these hidden causes.

SERIES K.

The soils employed in the preceding series for the study of ammonification were used also in the present series for the study of nitrification. The several soil portions, 100 gms. each, were arranged in three groups as heretofore. In Group *a* unsterilized portions of soil were mixed either with 100 mgs. of ammonium sulphate or 200 mgs. of dried blood. In Group *b* similar portions were previously sterilized, sterile ammonium sulphate or dried blood added, and were then inoculated with equal quantities of the same infusion from a fertile soil. In Group *c*, 100 gm. portions of this fertile soil were sterilized, supplied with ammonium sulphate or dried blood and inoculated with equivalent quantities of infusion from soils A to E. The soil portions of Group *a* were to express the influence of both the chemical and bacteriological factors; those of Group *b* the influence of the chemical factor and those of Group *c* the influence of the bacteriological factor.

The soil portions of Group *a* were kept in covered tumblers; those of Groups *b* and *c* in stoppered erlenmyer flasks. Sterile water was added from time to time in order to maintain optimum conditions as to moisture. All of the soil portions were kept in the incubator at 28° C. for a period of four weeks. At the end of that time the soils were leached, and nitrates and nitrites were determined colorimetrically. The following table shows the treatment of the several soil portions and also the amounts of nitrate and nitrite nitrogen found in each. The determination of the latter failed to supply information of any particular value:

GROUP *a*.

Soil No.	Soil Portion.	Addition.	Nitrate N Found.	Nitrite N Found.
A	91.....	0	Trace.	0.0760 mgs.
	92.....100 mgs.	$(NH_4)_2SO_4$.	"	0.0868 "
	93.....200 "	dried blood.	"	0.0608 "
B	94.....	0	4.000 mgs.	0.0808 "
	95.....100 "	$(NH_4)_2SO_4$.	4.125 "	0.0606 "
	96.....200 "	dried blood.	0.025 "	0.0720 "
C	97.....	0	8.000 "	0.0760 "
	98.....100 "	$(NH_4)_2SO_4$.	0.032 "	0.0768 "
	99.....200 "	dried blood.	0.100 "	0.0672 "
D	100.....	0	2.500 "	0.0615 "
	101.....100 "	$(NH_4)_2SO_4$.	10.000 "	0.1348 "
	102.....200 "	dried blood.	7.224 "	0.1012 "
E	103.....	0	0.125 "	0.0868 "
	104.....100 "	$(NH_4)_2SO_4$.	13.332 "	0.1012 "
	105.....200 "	dried blood.	Trace.	0.0782 "

GROUP *b*.

Soil No.	Soil Portion.	Addition.	Nitrate N Found.	Nitrite N Found.
A	106.....100 mgs.	$(NH_4)_2SO_4$.	Trace.	0.0820 mgs.
	107.....200 "	dried blood.	"	0.1000 "
B	108.....100 "	$(NH_4)_2SO_4$.	"	0.0520 "
	109.....200 "	dried blood.	"	0.0765 "
C	110.....100 "	$(NH_4)_2SO_4$.	4.050 mgs.	0.0600 "
	111.....200 "	dried blood.	0.020 "	0.1360 "
D	112.....100 "	$(NH_4)_2SO_4$.	4.160 "	0.1020 "
	113.....200 "	dried blood.	6.664 "	0.1280 "
E	114.....100 "	$(NH_4)_2SO_4$.	14.284 "	0.1216 "
	115.....200 "	dried blood.	0.085 "	0.1084 "

GROUP *c*.

Soil No.	Soil Portion.	Addition.	Nitrate N Found.	Nitrite N Found.
A	116.....100 mgs.	$(NH_4)_2SO_4$.	Trace.	0.0621 mgs.
	117.....200 "	dried blood.	0.500 mgs.	0.1428 "
B	118.....100 "	$(NH_4)_2SO_4$.	1.664 "	0.0800 "
	119.....200 "	dried blood.	1.920 "	0.1220 "
C	120.....100 "	$(NH_4)_2SO_4$.	0.552 "	0.0640 "
	121.....200 "	dried blood.	1.428 "	0.0960 "
D	122.....100 "	$(NH_4)_2SO_4$.	1.306 "	0.0805 "
	123.....200 "	dried blood.	0.652 "	0.0872 "
E	124.....100 "	$(NH_4)_2SO_4$.	1.818 "	0.1124 "
	125.....200 "	dried blood.	1.280 "	0.1064 "

Comparing the yields of nitrate nitrogen in the treated and untreated soil portions of Group *a* we find the following:

	Untreated.	*$(NH_4)_2SO_4$*	*Dried Blood.*
Soil A.....	Trace.	Trace.	Trace.
" B.....	4.000 mgs.	4.125 mgs.	0.025 mgs.
" C.....	8.000 "	0.032 "	0.100 "
" D.....	2.500 "	10.000 "	7.224 "
" E.....	0.125 "	13.332 "	Trace.

No nitrate was formed in the different portions of soil A, in the four weeks of the experiment. Under the same conditions soil B furnished 4.000 mgs. of nitrate nitrogen in the untreated portion, and 4.125 mgs. and 0.025 mgs. in the portions that had received ammonium sulphate and dried blood, respectively. Other differences fully as definite in one direction or another will be found also in the other soils. We see, therefore, that in the untreated soils the production of nitrate nitrogen ranged from a mere trace to 8.000 mgs.; in the soil portions receiving ammonium sulphate from a trace to 13.332 mgs.; and in the soil portions receiving dried blood, from a trace to 7.224 mgs. In soil C the production of nitrate was depressed by the ammonium sulphate; while in soils B, C and E it was depressed by the dried blood. Moreover, in all of the soils where the production of nitrates was considerable, the ammonium sulphate proved superior to the dried blood. It is not unlikely, of course, that in a period longer than four weeks the relations noted might have been appreciably modified; however, this does in nowise detract from the significance of the data before us.

Comparing similarily the yields of nitrate nitrogen in Groups *b* and *c*, we note the following:

	GROUP *b*.		GROUP *c*.	
	$(NH_4)_2SO_4$	*Dried Blood.*	*$(NH_4)_2SO_4$*	*Dried Blood.*
Soil A.....	Trace.	Trace.	Trace.	0.500 mgs.
" B.....	"	"	1.664 mgs.	1.920 "
" C.....	4.050 mgs.	0.020 mgs.	0.552 "	1.428 "
" D.....	4.160 "	6.664 "	1.306 "	0.652 "
" E.....	14.284 "	0.085 "	1.818 "	1.280 "

When sterilized and inoculated with infusion from fertile soil, soils A and B were found incapable of supporting nitrate formation during the experiment period. On the other hand, soils C, D

and E produced very considerable quantities of nitrate from the ammonium sulphate, while soil D produced 6.664 mgs. nitrate nitrogen also from the dried blood. Furthermore, soil E, which gave the highest yield also from the ammonium sulphate in Group *a*, gave the highest yield in Group *b*; and soil D, which gave the highest yield from the dried blood in Group *a*, gave the highest yield also in Group *b*. We must conclude, thus, that in the nitrification of ammonium sulphate and dried blood the chemical factor was paramount in soils A, D and E; and perhaps also in the other soils. This belief is strengthened by the results of Group *c*. We find here that with a chemical factor that is constant the variation in nitrate production is comparatively slight. In the case of the ammonium sulphate no nitrate was produced by the infusion from soil A, showing that in this particular soil both the chemical and bacteriological factors of nitrification were unsatisfactory.

Among the other soils E seemed to possess the most efficient bacteria for the nitrification of ammonium sulphate, and soil B, for the nitrification of dried blood. Other interesting differences are similarly presented by the data at hand, especially when we compare them with the corresponding data on ammonification in the preceding series. However, since the experiments under consideration are to a great extent preliminary, it would be wise not to draw definite conclusions until a greater mass of data is accumulated under varying experimental conditions.

THE EFFECTS OF SOLUBLE AND INSOLUBLE CARBOHYDRATES ON AMMONIA FORMATION IN SOILS AND CULTURE SOLUTIONS.

Earlier experiments have demonstrated[1] that in the presence of a soluble carbohydrate, *e. g.*, dextrose, the formation (or at least the accumulation) of ammonia from peptone was depressed. It was further demonstrated[2] that in the soil portions where peptone alone was used the number of bacteria was made larger than that in the soil portions where both peptone and dextrose were employed. Insoluble carbohydrates like cellulose depressed ammonia formation and the numbers of bacteria very slightly or not at all. On the other hand, it was shown in the present paper that addi-

[1] Lipman and Brown, N. J. Sta. Report, 1908, pp. 100–105.
[2] 1 c. p. 105.

tions of dextrose and sodium citrate to soils kept in the greenhouse led, in the course of time, to an enormous increase in the numbers of bacteria in these soils. Moreover, when the soils under consideration were employed for inoculation into peptone solutions, or for mixing with definite quantities of peptone, the greater number of bacteria present in them did not result in ammonification more intense than that caused by portions of similar soils that had received no applications of dextrose. It therefore appeared from these experiments that the ammonification of peptone may be temporarily retarded by the presence of soluble carbohydrates through their unfavorable influence on the development of soil bacteria for at least a brief period. It also appeared that the temporary depression in the growth of the bacteria was followed by more intense development. Since, however, the comparatively large number of bacteria thus developed did not produce more ammonia from peptone than was produced in similar soil portions with smaller numbers of bacteria, the subject was further investigated and it was found that the ammonification of peptone is to some extent analogous to the ammonification of urea in that it may be accomplished by enzyme action. It followed, therefore, that beyond a certain point soils with a large number of bacteria could ammonify no more peptone than soils with a small number of bacteria, it being assumed, of course, that the chemical factors were the same. As a result of these inquiries it was determined to investigate the matter further, and to test the effect of soluble and insoluble carbohydrates on nitrogenous materials that undergo ammonification less readily than peptone. There were selected for this purpose dried blood and cottonseed meal, and their ammonification was studied in the soil itself as well as in suspensions in water.

SERIES L.

A quantity of fertile soil from the College Farm was carefully sifted and mixed with an equal weight of pure quartz sand. One hundred gram portions of this mixture were placed in covered tumblers, mixed with the different materials used, brought to an optimum moisture content by the addition of sterile water, and placed in the incubator. Ammonia determinations were made at

the end of seven days by transferring the soil to copper flasks, adding water and magnesia, distilling off the ammonia, and titrating against standard solutions in the usual way. The treatment of the several soil portions and the amounts of ammonia found are recorded in the following table:

Soil Portion No.	*Additions.*	*Ammonia N Found.*	*Average.*
1	5 gms. dried blood.	167.88 mgs.	
2	" " "	153.97 "	160.92 mgs.
3	" " " + 0.5 gm. dextrose.	165.44 "	
4	" " " " " "	129.39 "	147.41 "
5	" " " + 1.0 " "	155.33 "	
6	" " " " " "	157.41 "	156.37 "
7	" " " + 3.0 " "	151.86 "	
8	" " " " " "	168.58 "	160.22 "
9	" " " 0.5 " starch.	131.66 "	
10	" " " " " "	156.91 "	144.28 "
11	" " " 1.0 " "	142.11 "	
12	" " " " " "	137.75 "	139.93 "
13	" " " 3.0 " "	40.05 "	
14	" " " " " "	36.57 "	38.31 "
15	" " " 0.5 " filter paper.	159.35 "	
16	" " " " " " " "	164.40 "	161.87 "
17	" " " 1.0 " " "	179.37 "	
18	" " " " " " " "	180.07 "	179.72 "
19	" " " 3.0 " " "	194.70 "	
20	" " " " " " " "	190.52 "	196.76 "
21	" cottonseed meal.	109.71 "	
22	" " "	125.38 "	117.54 "
23	" " " + 0.5 gm. dextrose.	116.68 "	
24	" " " " " "	117.20 "	116.94 "
25	" " " 1.0 " "	97.70 "	
26	" " " " " "	103.79 "	100.74 "
27	" " " 3.0 " "	2.79 "	
28	" " " " " "	4.88 "	3.83 "
29	" " " 0.5 " starch.	115.98 "	
30	" " " " " "	113.54 "	114.76 "
31	" " " 1.0 " "	90.56 "	
32	" " " " " "	69.83 "	80.19 "
33	" " " 3.0 " "	23.34 "	
34	" " " " " "	17.07 "	20.20 "
35	" " " 0.5 " filter paper.	130.09 "	
36	" " " " " " " "	136.18 "	133.13 "
37	" " " 1.0 " " "	142.10 "	
38	" " " " " " " "	142.98 "	142.54 "
39	" " " 3.0 " " "	156.68 "	
40	" " " " " " " "	148.90 "	152.79 "

Making due allowance for the rather wide variations in some of the duplicates, we find, in the above data, some definite information concerning the influence of soluble and insoluble carbohydrates on the ammonification of dried blood and of cottonseed meal. We note that in the soil portions where dried blood alone was used the average amount of ammonia nitrogen produced in seven days was 160.92 mgs. The addition of 0.5 gm. of dextrose apparently reduced this to 147.41 mgs. However, this amount is smaller than it should have been, because of the comparatively small yield of ammonia nitrogen in soil portion 4, viz., 129.39 mgs. Furthermore, the yields of ammonia nitrogen in the soil portions that had received additions of one gm. and of three gm. of dextrose were 156.37 mgs. and 160.22 mgs., respectively. Hence we must conclude that in this particular experiment the addition of dextrose up to three gms. per 100 gms. of soil and five gm. of dried blood did not depress the ammonification of the latter.

But when we come to consider the effect of starch under the same conditions we note a distinct depression in ammonia accumulation. With 0.5 gm. of starch the amount of ammonia nitrogen was reduced to 144.28 mgs., with one gm. to 139.93 mgs., and with three gms. to only 38.31 mgs. Why starch, less soluble than dextrose, should have had a more depressing effect than the latter is not clear, particularly since in peptone solutions dextrose proved more efficient than starch in depressing the accumulation of ammonia.

In examining the corresponding results secured from the use of filter paper we find an increase, rather than a decrease in the amount of ammonia nitrogen present in the several soil portions. With 0.5 gm. of filter paper there were secured 161.87 mgs. of ammonia nitrogen—that is, about the same amount found in the soil portions in which dried blood alone was used. With one and three gms. of filter paper the amounts of ammonia nitrogen found were 179.72 mgs. and 196.76 mgs., respectively. It still remains to be determined whether the favorable influence of filter paper on ammonia accumulation in the soil was due to mechanical or chemical effects. A similar favorable effect of filter paper was observed by one of us in the study of nitrogen-fixation by Azotobacter in mannite solutions.[1]

[1] N. J. Sta. Rep. 1903, p. 262.

Comparing the ammonification of cottonseed meal under the same conditions we observe relations analogous to those noted in the case of the dried blood. However, the depressing effect of dextrose was quite pronounced with the cottonseed meal, where none was observed with the dried blood. It will be noted that one gm. of dextrose caused a decrease in the amount of ammonia nitrogen found in the soil from 117.54 mgs. to 100.74 mgs., while three gms. of dextrose decreased it to 3.83 mgs. Where starch was used in place of the dextrose the depression was even more marked with one gm. of material, but not quite as marked with three gms. On the other hand, the use of filter paper resulted in an increase, instead of a decrease in the amount of ammonia found in the soil portions. This agrees entirely with the results in the corresponding soil portions that had received additions of dried blood. As to the depression of ammonification by dextrose in the case of the cottonseed meal, as contrary to the results obtained with dried blood, it must be assumed that the fat and crude fiber of the former may account for these differences. At any rate there is reason to suspect that the carbon nitrogen ratio is of moment in the rate of ammonification of nitrogenous materials, and that the modification of this ratio by soluble carbohydrates, or by other soluble carbon compounds, may lead to changes in the numbers and species of the micro-organisms in the soils or culture solutions.

SERIES M.

Several weeks after the results of Series L were secured a similar series was started with 100 gm. portions of fertile soil from an alfalfa field. The several soil portions were kept in covered beakers at optimum moisture conditions for three days when peptone was employed, and for seven days when dried blood and cottonseed meal were employed. The following table shows the treatment of each soil portion as well as the amounts of ammonia nitrogen found:

Soil Portion No.	Additions.	Ammonia N Found.	Average.
1	0.5 gm. peptone.	46.20 mgs.	
2	" "	46.90 "	46.55 mgs.
3	" " + 1.0 gm. dextrose.	18.72 "	
4	" " " "	15.75 "	17.23 "
5	" " + 3.0 gm. "	1.75 "	
6	" " " "	4.02 "	2.88 "
7	5.0 gms. dried blood.	139.47 "	
8	" " "	145.42 "	142.44 "
9	" " " + 1.0 gm. dextrose.	155.05 "	
10	" " " " "	148.05 "	151.55 "
11	" " " 3.0 gm. "	155.92 "	
12	" " " " "	144.20 "	150.06 "
13	" cottonseed meal.	141.22 "	
14	" " "	141.57 "	141.40 "
15	" " " + 1.0 gm. dextrose.	137.20 "	
16	" " " " "	135.97 "	136.58 "
17	" " " 3.0 gm. "	123.55 "	
18	" " " " "	116.37 "	119.96 "
19	2.0 gms. dried blood.	73.67 "	
20	" " "	69.12 "	71.39 "
21	" " " + 1.0 gm. dextrose.	84.00 "	
22	" " " " "	76.12 "	80.06 "
23	" " " 3.0 gm. "	47.42 "	
24	" " " " "	43.40 "	45.41 "

The data recorded above confirm those secured in preceding series. We note here again that the ammonification of peptone was depressed by one gm. of dextrose and still more so by three gms. of dextrose. We find, also, that the smaller amount of dextrose stimulated, rather than depressed the ammonification of dried blood, while the larger amount of dextrose still failed to depress it. Similarly the ammonification of the cottonseed meal was depressed by both the smaller and the larger amount of dextrose, although the depression was by no means as marked here as it was in Series L, owing probably to differences in the soils employed.

Aside from confirming the data of Series L, the results of the present series serve to furnish additional information on the relation of soluble carbohydrates to ammonification of dried blood. It will be noted that soil portions 19 to 20 correspond to soil portions 7 to 12 except that they contain two gms. instead of five gms. of dried blood. This difference establishes a different ratio of dextrose to dried blood and serves to show whether the depression in ammonification bears any relation to this ratio. We find, accordingly, that in soil portions 19 and 20 where dried blood alone was employed, the average amount of ammonia nitrogen produced was 71.39 mgs. With the smaller amount of dextrose this was

increased to 80.06 mgs., but with the larger amount of dextrose there was a distinct decrease to 45.41 mgs. It appeared, therefore, that under any given soil conditions the ammonification of nitrogenous materials may depend not only on the chemical and mechanical compositions of the latter, but also on the quantity and quality of the non-nitrogenous organic substances present. It seems thus that the very marked depression of ammonia formation from peptone was due both to its solubility and to the comparatively small amount of material used, viz., 0.5 gm. per 100 gms. of soil.

SERIES N.

As a further confirmation of these results, still another series was started a month later. The arrangement of Series M was duplicated in all particulars, except that one gm. of calcium carbonate was mixed with each soil portion. This was done in order to determine whether the acids produced in the decomposition of the carbohydrates were responsible for decreased ammonia production. The treatment of the several soil portions was as follows:

Soil Portion. No.	*Additions.*	*Ammonia N Found.*	*Average.*
1	0.5 gm. peptone.	42.87 mgs.	
2	" "	42.52 "	42.69 mgs.
3	" " + 1.0 gms. dextrose.	16.10 "	
4	" " " "	17.85 "	16.97 "
5	" " + 3.0 gms. "	2.45 "	
6	" " " "	2.62 "	2.53 "
7	5.0 gms. dried blood.	250.95 "	
8	" " "	248.50 "	249.72 "
9	" " " + 1.0 gm. dextrose.	249.37 "	
10	" " " " "	230.12 "	239.74 "
11	" " " + 3.0 gm. "	212.62 "	
12	" " " " "	205.27 "	208.94 "
13	" cottonseed meal.	121.10 "	
14	" " "	122.67 "	121.88 "
15	" " " + 1.0 gm. dextrose.	98.52 "	
16	" " " " "	95.72 "	97.12 "
17	" " " + 3.0 gm. "	69.47 "	
18	" " " " "	70.35 "	69.91 "
19	2.0 gms. dried blood.	150.85 "	
20	" " "	148.22 "	149.53 "
21	" " " + 1.0 gm. dextrose.	139.12 "	
22	" " " " "	136.15 "	137.63 "
23	" " " + 3.0 gm. "	99.75 "	
24	" " " " "	101.32 "	100.53 "

The amounts of ammonia nitrogen found in the soil portions containing peptone correspond very closely to those found in the preceding series. Notwithstanding the fact that different lots of the same soil were used and that the two experiments were carried out at different times, the results secured are almost identical. In Series M the average amounts of ammonia nitrogen produced were 46.55 mgs., 17.23 mgs. and 2.88 mgs., respectively. In Series N they were 42.69 mgs., 16.97 mgs. and 2.53 mgs., respectively. The lime employed in the latter series did not affect these results to any appreciable extent. We are thus furnished with further evidence concerning the readiness with which peptone is ammonified in the soil, the bacterial activities being so intense as to accomplish maximum results in a comparatively short time.

But when we come to compare the ammonification of the dried blood in the two series we find some striking differences. In Series M the amounts of ammonia nitrogen produced from five gms. of dried blood were 142.44 mgs., 151.55 mgs. and 150.06 mgs., respectively. In Series N the corresponding amounts were 249.72 mgs., 239.74 mgs. and 208.94 mgs., respectively. It is clear, therefore, that the presence of calcium carbonate intensified the ammonification of dried blood in a very striking manner. Furthermore, in the presence of dextrose a more or less pronounced depression in ammonification occurred in the soil portions with five gms. of dried blood. With one gm. of dextrose the amount of ammonia found was reduced from 249.72 mgs. to 239.74 mgs., while with three gms. of dextrose it was reduced to 208.94 mgs. On the contrary, in Series M, an increase was noted in the corresponding soil portions. Similarly, in the soil portions with two gms. of dried blood, the presence of calcium carbonate intensified ammonification, and the depression in ammonia accumulation was, on the whole, fully as pronounced as it was in Series M.

While the presence of calcium carbonate in Series N stimulated the ammonification of dried blood it failed to do so in the case of the cottonseed meal. In Series M the amounts of ammonia nitrogen produced were 141.40 mgs., 136.58 mgs. and 119.96 mgs., respectively. In Series N they were 121.88 mgs., 97.12 mgs. and 69.91 mgs., respectively. It is not clear why calcium carbonate, which usually intensifies the ammonification of nitrogenous materials, should have depressed the ammonification of cottonseed meal.

SERIES O.

The results of the foregoing series were secured in 100 gm. portions of soil. The results recorded in the present series were secured in 100 c.c. portions of tap water in which the nitrogenous materials were either dissolved or suspended. The amounts employed in each 100 c.c. portion were one gm. in the case of peptone, and five gms. in the case of dried blood and cottonseed meal. The amounts of dextrose used consisted of one gm. and three gms., respectively. The several flasks and contents were sterilized in the autoclave and, on cooling, were each inoculated with twenty c.c. of suspension representing ten gms. of fertile soil derived from an alfalfa field at the College Farm. The cultures were kept in the incubator at room temperature. Ammonia determinations were made in the usual way at the end of three days in the case of the peptone, and at the end of seven days in the case of the dried blood and cottonseed meal. The treatment of each culture and the amounts of ammonia nitrogen found are recorded in the following table:

Culture No.	*Additions.*	*Ammonia N Found.*	*Average.*
1	100 c.c. tap water, 1.0 gms. peptone.	79.97 mgs.	
2	" " " "	91.00 "	85.48 mgs.
3	" " " " + 1.0 gm. dextrose.	3.50 "	
4	" " " " " "	4.02 "	3.76 "
5	" " " " + 3.0 gm. "	0.17 "	
6	" " " " " "	0.00 "	0.08 "
7	" " 5.0 gms. dried blood.	81.02 "	
8	" " " " "	62.82 "	71.92 "
9	" " " " " + 1.0 gm. "	27.30 "	
10	" " " " " " "	22.05 "	24.67 "
11	" " " " " + 3.0 gm. "	14.70 "	
12	" " " " " " "	10.15 "	12.42 "
13	" " 5.0 gms. cottonseed meal.	25.02 "	
14	" " " " "	29.05 "	27.03 "
15	" " " " " + 1.0 gm. "	14.17 "	
16	" " " " " " " "	18.55 "	16.36 "
17	" " " " " + 3.0 gm. "	2.62 "	
18	" " " " " " " "	2.97 "	2.79 "

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It will be noted in the above table that the variations in the duplicate portions are rather wider than they were, in many instances, in the soil portions of the preceding series. For all that the depression due to the dextrose added was very definite. In the case of the peptone one gm. of dextrose reduced the amount of ammonia nitrogen in the culture solution from 85.49 mgs. to 3.76 mgs., while the three gms. of dextrose caused the disappearance of all but traces of the ammonia nitrogen. In the case of the dried blood the smaller amount of dextrose reduced the average amount of ammonia nitrogen in the liquid from 71.92 mgs. to 24.67 mgs. The larger amount of dextrose reduced it to 12.42 mgs. The depression is therefore unmistakable even here, which is contrary to the results secured with the soil portions of Series M and N. Finally, in the case of the cottonseed meal, the smaller amount of dextrose reduced the amount of ammonia in the liquid from 27.03 mgs. to only 2.79 mgs. It seems, thus, that the ammonification of dried blood and of cottonseed meal is readily checked by soluble carbohydrates when the nitrogenous materials are suspended in sterile tap water, instead of being mixed with 100 gms. of soil. Whether this checking be due to the less plentiful supply of oxygen in the culture solutions; to the less thorough removal of the injurious products of metabolism, or to some other cause cannot be stated definitely. Meanwhile, another series, similar to the present, was arranged to serve not only as a check on the results just noted, but also to ascertain the possible modifying influence of calcium carbonate.

SERIES P.

The peptone, dried blood, cottonseed meal and dextrose were employed in a manner already described in Series O. The calcium carbonate was added at the rate of one gm. per 100 c.c. of solutions. After sterilization in the autoclave, and cooling, each portion was inoculated with twenty c.c. infusion equivalent to ten gm. of soil. Ammonia was determined in the peptone cultures at the end of three days, and in the dried blood and cottonseed meal cultures at the end of seven days. The amounts of ammonia nitrogen found in the cultures were as follows:

Culture No.	*Additions.*	*Ammonia N Found.*	*Average.*
1	100 c.c. tap water, 1 gm. $CaCO_3$, 1.0 gms. peptone.	49.35 mgs.	
2	" " " " " "	54.95 "	52.15 mgs.
3	" " " " " " + 1.0 gm. dextrose.	4.02 "	
4	" " " " " " " "	3.15 "	3.58 "
5	" " " " " " + 3.0 gm. "	3.50 "	
6	" " " " " " " "	5.25 "	4.37 "
7	" " " 5.0 gms. dried blood.	47.77 "	
8	" " " " " "	48.65 "	48.21 "
9	" " " " " " + 1.0 gm. "	21.52 "	
10	" " " " " " " "	22.40 "	21.96 "
11	" " " " " " + 3.0 gm. "	6.12 "	
12	" " " " " " " "	7.00 "	6.56 "
13	" " " 5.0 gms. cottonseed meal.	36.75 "	
14	" " " " " "	31.50 "	34.12 "
15	" " " " " + 1.0 gm. "	9.45 "	
16	" " " " " " "	9.45 "	9.45 "
17	" " " " " + 3.0 gms. "	2.80 "	
18	" " " " " " "	1.40 "	2.10 "

Ammonification was depressed, in the present series, in every instance where dextrose was used. The facts previously noted are thus amply confirmed. It should be noted here, however, that in the cultures receiving no additions of dextrose the ammonification of the peptone and dried blood was further advanced in Series O than it was in the present series because of the lower temperature at which the latter experiment was carried out. As to the influence of the calcium carbonate we again note a tendency toward more intense ammonification, at least in the cultures to which one gm. of dextrose was added. On the other hand, in the case of the cottonseed meal the calcium carbonate depressed ammonification in the cultures to which one gm. of dextrose was added. Everything considered, therefore, we may conclude that the ammonification of peptone is depressed in the soil, and even more markedly in solutions, by the presence of certain proportions of dextrose and of other soluble carbohydrates. But in so far as the more inert nitrogenous materials are concerned, the depression does not occur as readily, although it may also be brought about by gradually increasing amounts of soluble carbohydrates. When these materials are suspended in water their ammonification is retarded more readily than it is in the soil itself. It may be added here that in so far as dried blood and similar materials are concerned the presence of small amounts of dextrose may lead to an increase rather than to a decrease in the amount of ammonia formed when ammonification is taking place in the soil itself. In culture solutions this is true to a more limited extent.

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Taking the data just considered as they stand, the question naturally arises as to the cause of the depressed ammonification in the presence of dextrose, starch, &c. The fact that less ammonia is found in the soil portions or cultures that had received additions of dextrose does not show, of course, that less ammonia was formed. We know that ammonia is used as a source of food by many species and its accumulation in the soil under ordinary field conditions is prevented for this very reason. Now, when a proportionately large amount of sugar or of other soluble, non-nitrogenous organic substances, is present in the soil material is furnished for the production of organic acids which in turn react both on the rate of multiplication of the soil bacteria and the species relationships. Hence, we may properly ask whether the presence of dextrose depresses ammonification by partly or wholly suppressing the ammonia forming bacteria, or by favoring the rapid multiplication of the ammonia consuming bacteria. In the one case less ammonia will be formed; and less will be found, therefore, in the soil or culture solution. In the other case as much or more will be formed, as in similar cultures receiving no additions of dextrose, yet less will be found because of the more intense consumption. It is evident that more definite information in this direction may be secured by inoculating equal amounts of soil, previously sterilized, with a pure culture of some ammonifying species. In accordance with this thought the following series was arranged.

AMMONIA FORMATION BY B. MYCOIDES AS AFFECTED BY THE PRESENCE OF DEXTROSE.

SERIES R.

Ammonification was carried on here in 100 gm. portions of material containing fifty gms. fertile soil and fifty gms. of white sand. The mechanical character of this mixture was such as to allow a rapid multiplication of ærobic bacteria. Each portion received also an addition of one gm. of calcium carbonate, aside from the peptone, dried blood, cottonseed meal and dextrose employed. After sterilization in the autoclave and cooling each portion was inoculated with one c.c. of a three days' old culture of *B. mycoides.*

The several soil portions were kept in the incubator at 28° C., at optimum moisture conditions. Ammonia determinations were made at the end of ten days. The treatment of the different portions and the amounts of ammonia nitrogen found are recorded in the following table:

Soil Portion No.	Additions.	Ammonia N Found.	Average
1	1.0 gm. peptone.	81.37 mgm.	
2	" "	91.17 "	86.27 mgm.
3	" " + 1.0 gm. dextrose.	84.17 "	
4	" " " "	78.57 "	81.37 "
5	" " + 3.0 gm. "	53.55 "	
6	" " " "	54.25 "	53.90 "
7	5.0 gms. dried blood.	60.02 "	
8	" " "	72.45 "	66.23 "
9	" " " - 1.0 gm. dextrose.	125.65 "	
10	" " " " "	131.42 "	128.53 "
11	" " " - 3.0 gm. "	147.52 "	
12	" " " " "	152.95 "	150.23
13	" cottonseed meal.	96.07 "	
14	" " "	91.87 "	93.97 "
15	" " " - 1.0 gm. dextrose	90.65 "	
16	" " " " "	92.77 "	91.71 "
17	" " " - 3.0 gm. "	76.12 "	
18	" " " " "	86.20 "	81.46
19	1.0 gm. peptone - 3.1 gms dextrose 5% (all)	6.07 "	
20	" " " " "	76.75 "	91.41 "
21	5.0 gms. dried blood - 3.1 gms dextrose 5% (all)	221.47*	
22	" " " " "	74.80 "	148.13
23	" cottonseed meal.	74.45 "	
24	" " " " "	77.7	76.1

In examining the [illegible] that *B.* [illegible] suitable [illegible] no ad[illegible] 86.27 [illegible] from the [illegible] Where [illegible] mistake [illegible] from the [illegible] 81.37 [illegible] the [illegible] manner [illegible] amo[illegible]

* See [illegible]

with three gms. to 150.23 mgs. In the case of the cottonseed meal there was a comparatively small reduction in the amount of ammonia accumulated in the soil when dextrose was present. To sum it up, therefore, dextrose decreased the production of ammonia by *B. mycoides* from peptone and cottonseed meal, and increased it to a very marked extent where dried blood was employed. When the calcium carbonate was left out there was no very considerable difference in the production of ammonia from peptone and cottonseed meal in the presence of three gms. of dextrose. In the case of the dried blood, however, the results are not as definite either on account of some error in the analytical work, or on account of abnormal ammonification in soil portion 21. Hence the amount of ammonia nitrogen found in this soil portion was not included in the average. Taking the yield of soil portion 22 we observe that it is somewhat lower than that in the corresponding soil portions 11 and 12. This result may have been expected, however, because of the stimulating effect of calcium carbonate on ammonification as observed in the preceding series. We thus come to find that a pure culture of *B. mycoides* may give results very much akin to those secured with mixed cultures of soil bacteria. For this reason we cannot assume that the smaller accumulation of ammonia in the soil or culture solutions caused by the presence of dextrose is due to changes in species. We can assume, however, that the smaller amount of ammonia found may be due to decreased production or increased consumption. In the first case it may be supposed that the dextrose retarded the multiplication of the bacteria, or lessened the efficiency of the individual cell. In the second case we may assume that, thanks to the presence of the dextrose, the bacteria not only multiplied more rapidly and produced more ammonia, but used up a larger proportion of the latter and thus retarded its accumulation. While it is still too early to draw definite conclusions as to the true cause of the depressed ammonification under the conditions named, it may be pointed out here that in the case of peptone, at least, there may be a retarded multiplication of bacteria, brought about by dextrose and similar substances at the beginning of the experiment. Later on, however, the soil portions supplied with dextrose are found to contain a much larger number of bacteria than similar soil portions without dextrose. How long the depressing effect of different

amounts of dextrose may last under varying soil conditions still remains to be determined. With more exact data in this direction we shall be able to understand why it was that the ammonification of dried blood was enhanced rather than depressed by dextrose in a number of instances, and also whether the assumed importance in this connection of the carbon-nitrogen ratio is well founded.

COMPARATIVE TESTS ON THE AMMONIFICATION AND NITRIFICATION OF NITROGENOUS MATERIALS.

SERIES S.

Attempts to determine the relative availability of nitrogenous fertilizers by measuring the amounts of nitrate formed from them under definite experimental conditions, have been made by different investigators. In consequence of these investigations it is now well known that nitrate formation from organic substances bears a direct relation to the proportion of nitrogen contained in them, and, in general, to the ease with which they will undergo decomposition. A much larger amount of work on the same subject has been done from the crop standpoint; that is, the relative availability of nitrogenous substances has been determined not by measuring the quantity of nitrate formed from them under definite experimental conditions, but by measuring the amounts of nitrogen furnished by each to the crop, as shown by analyses of the latter.

In view of the fact that nitrate formation from organic substances is preceded by ammonia formation, it is proper to assume that nitrification is more or less directly affected by ammonification. Substances that will ammonify readily should also nitrify readily, and *vice versa*. Nevertheless, it is theoretically possible that the relative ammonification of a group of nitrogenous substances need not correspond exactly to their relative nitrification. It therefore appeared advisable to make a series of tests on the relative ammonification and nitrification of a number of such substances. It was hoped, also, that among other things, these tests would give some indication as to the practicability of measuring the relative availability of nitrogenous materials by their ammoni-

fication, as well as their nitrification. It is to be added here that the experiments recorded below are hardly more than preliminary, and will be continued in the future.

The nitrogenous materials in these tests were mixed in each case with 100 gms. of soil and one gm. of calcium carbonate. The soils themselves were kept at their optimum moisture content, viz., 18 per cent. In the ammonification tests additional amounts of water were supplied so as to provide in each case organic matter containing 75 per cent. of water. For instance, when five gms. of dried blood were employed there were added fifteen c.c. of water aside from that added to the soil itself; when approximately thirty gms. of dried manure were used the amount of water added was about ninety c.c., &c. Thanks to this method it was possible to maintain uniform moisture conditions in the several soil portions. Both in the nitrification and ammonification tests the materials were added so as to supply equal amounts of nitrogen. The nitrogen content of the different substances was as follows:

Ammonium sulphate	20.30	per cent.
Calcium cyanamid	17.64	" "
Dried blood	10.76	" "
Concentrated tankage	12.63	" "
Ground fish	7.90	" "
Cottonseed meal	6.40	" "
Bone meal	1.35	" "
Solid and liquid manure, fresh	3.07	" "
Solid manure, fresh	2.20	" "
Solid and liquid manure, leached	1.72	" "
Horse manure	1.81	" "

Ammonia determinations were made at the end of six days, and nitrate determinations at the end of four weeks in the corresponding series. The amounts of material used and those of ammonia and nitrate nitrogen found were as follows:

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Soil. Portion No.	*Additions.*	*Ammonia N Found.*	*Average.*
1	5 gms. dried blood	91.51 mgs.	
2	" " "	88.67 "	90.09 mgs.
3	4.26 gms. concentrated tankage	312.95 "	
4	" " "	297.99 "	305.47 "
5	6.81 gms. ground fish	247.43 "	
6	" " "	260.03 "	253.73 "
7	8.41 gms. cottonseed meal	32.13 "	
8	" " "	21.42 "	26.77 "
9	39.85 gms. bone meal	78.43 "	
10	" "	102.06 "	90.24 "
11	17.52 gms. solid and liquid manure, fresh	180.49 "	
12	" " " "	170.41 "	175.45 "
13	24.45 gms. solid manure, fresh	28.19 "	
14	" " " "	29.77 "	28.98 "
15	31.28 gms. solid and liquid manure, leached	62.37 "	
16	" " " " "	62.37 "	62.37 "
17	29.72 gms. horse manure	40.00 "	
18	" " "	36.85 "	38.42 "

Soil Portion No.	*Additions.*	*Nitrate N Found.*	*Average.*	*Increase over the Untreated Portions.*
1	0	5.332 mgs.		
2	0	5.332 "	5.33 mgs.	
3	100 mgs. ammon. sulphate	21.332 "		
4	" " "	20.720 "	21.26 "	15.928 mgs.
5	115 mgs. calcium cyanamid	*5.48 "		
6	" " "	16.00 "	16.00 "	10.668 "
7	189 mgs. dried blood	10.56 "		
8	" " "	10.71 "	10.63 "	4.298 "
9	161 mgs. concentrated tankage	16.21 "		
10	" " "	16.19 "	16.20 "	10.868 "
11	257 mgs. ground fish	10.75 "		
12	" " "	10.53 "	10.64 "	5.318 "
13	317 mgs. cottonseed meal	10.46 "		
14	" " "	12.65 "	11.55 "	6.218 "
15	1504 mgs. bone meal	7.74 "		
16	" " "	7.92 "	7.83 "	2.498 "
17	661 mgs. solid and liquid manure, fresh.	7.80 "		
18	" " " " "	8.00 "	7.90 "	2.568 "
19	923 mgs. solid manure, fresh	7.22 "		
20	" " " "	7.29 "	7.25 "	1.118 "
21	1180 mgs. solid and liquid manure, leached,	6.16 "		
22	" " " "	6.32 "	6.24 "	0.908 "
23	1122 mgs. horse manure	7.08 "		
24	" " "	7.14 "	7.11 "	1.778 "

* Not included in the average.

As is shown in the above table considerable quantities of nitrogen were split off either in the form of ammonia or of nitrate. Small quantities of nitrites were also found in the nitrification series, yet they were quite uniform, and so insignificant in amount as to make it unnecessary to record them here. The proportion of the amounts of nitrogen found as ammonia or nitrate, to the amounts applied may serve as an indication of the relative availability of the different materials. These proportions, designated as nitrogen recovered, are shown in the following table:

PER CENT. OF NITROGEN RECOVERED.

	Ammonification Series.	*Nitrification Series.*
Ammonium sulphate	.....	78.47
Calcium cyanamid	.....	52.56
Dried blood	16.74	26.13
Concentrated tankage	56.66	53.54
Ground fish	47.16	26.15
Cottonseed meal	4.95	30.64
Bone meal	16.65	12.31
Cow manure, solid and liquid, fresh	32.60	12.67
Cow manure, solid, fresh	5.39	9.46
Cow manure, solid and liquid, leached	11.59	4.48
Horse manure	7.14	8.76

In comparing the figures before us we should remember that ammonia was determined at the end of six days and nitrate at the end of four weeks. There is no doubt that two or three days more would have not only increased the amounts of ammonia produced in the ammonification series, but would have modified to some extent the relative recovery. It is also true that another week or two in the nitrification series would have resulted in slight modifications in the relative availability of the different materials as measured by nitrate production. For all that the results are of considerable interest even as they are. We note, for instance, that concentrated tankage, ground fish, solid and liquid manure, fresh, dried blood and bone meal in the order given yielded the largest amounts of ammonia and concentrated tankage, cottonseed meal, ground fish, dried blood and solid and liquid manure, fresh, in the order given yielded the largest amounts of nitrate. Concentrated tankage heads the list, in each case, while three others are also found in each group. The surmise, therefore, that easily ammonifiable

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substances are also easily nitrifiable is borne out by the present data. Nevertheless, there are some discrepancies that are of considerable moment. It will be observed that next to concentrated tankage, cottonseed meal proved the most easily nitrifiable of the organic materials. In the ammonification series, on the other hand, cottonseed meal was found to possess a low rate of availability. In view of the experiments recorded above as to the depressing effect of soluble carbohydrates on ammonification, the low rate of availability of cottonseed meal in the ammonification series is not without interest.

In comparing further the relative availabilities of the same materials in the ammonification and nitrification series it should be remembered that small quantities of material are transformed more thoroughly and more efficiently in the soil than larger quantities. Hence, the relations in the two series are somewhat different in this respect. It is also likely that in different soils the relations found in the two series would be modified. With that much admitted, however, it is instructive to note how rapidly some substances may be broken down by bacteria. We find, for instance, that in the ammonification series 56.66 per cent. of the nitrogen contained in the concentrated tankage was changed to ammonia in six days. Similarly, 47.16 per cent. and 32.60 per cent. in the ground fish and the solid and liquid manure, fresh, respectively, were transformed into ammonia. On the contrary, the three other samples of manure proved to have a low rate of availability, both in the ammonification and nitrification series. It should be noted here, however, that the samples of manure employed in the present experiments had been previously dried on the water bath and kept in a dry state for some time. Naturally, the drying reduces the availability of nitrogenous materials, hence the returns, as noted above, are smaller than they would have been had the manures in question been used in a fresh condition. It may be of interest to cite here the results secured with similar samples of manure in our cylinder experiments extending over a period of ten years. It was found in these experiments[1] that solid manure, fresh, solid and liquid, fresh, and solid and liquid, leached, gave recoveries of 22.31 per cent., 32.91 per cent. and 26.75 per cent., respectively, in ten periods, in which two rotations of corn, oats, wheat and grass had

[1] Bull. 221, N. J. Exper. Sta., p. 23.

been completed. The manure was applied annually and the figures given above represent, therefore, the cumulative effect of the manure residues. In single seasons much lower returns have been secured in a number of instances. Thus the corresponding recoveries in the corn crop of 1908 were[1] 11.20 per cent., 17.55 per cent. and 11.50 per cent., respectively. On the whole, it is not unlikely that the methods outlined above will prove very convenient not only for measuring the relative availability of nitrogenous fertilizers, but also for determining the availability of the nitrogen in humus derived from different soils.

NITRATE FORMATION AS AFFECTED BY SOIL VOLUME.

SERIES T.

In studying the relative availability of nitrogenous materials as shown by nitrate formation, the factor relating to *distribution*, or what is substantially the same thing, the ratio of the soil volume to that of the substance to be nitrified, is often of considerable moment. Everything being equal, a nitrogenous fertilizer that is evenly distributed in the soil and scattered through a larger bulk of it, will undergo ammonification and nitrification more rapidly and more uniformly than a similar material not properly distributed. It has already been shown that the amount of ammonia produced from a given quantity of dried blood varies within certain limits with the amount of soil in which the dried blood is distributed. In the case of peptone this relation did not hold good because of its unstable character and the readiness with which it may be transformed into ammonia. It appeared desirable, therefore, to determine whether nitrate formation, like ammonification, would be affected by variations in the soil volume. Quantities of soil weighing twenty-five gms., fifty gms. and 100 gms. were used in this experiment, and the additions consisted of 100 mgs. ammonium sulphate, 500 mgs. of dried blood, or 500 mgs. of cottonseed meal. Calcium carbonate was added to each soil portion at the rate of one gm. per 100 gms. of soil. Optimum

[1] Voorhees and Lipman, Experiments on the Utilization of Nitrogen in Fertilizer Materials, Journ. Industrial and Engineering Chemistry, vol. I (1909), p. 397.

moisture conditions and a temperature of 25 to 26° C. were maintained for six weeks. At the end of that time nitrate nitrogen was determined in each soil portion with the following results:

Soil Portion No.	*Additions.*	*Nitrate N Found.*	*Average.*	*Increase over Untreated Portions.*
1...	25 gms. soil + 0.25 gm. $CaCO_3$	3.334 mgs.		
2...	" " " "	3.500 "	3.417 mgs.	
3...	" " " " + 100 mgs. $(NH_4)_2SO_4$	10.000 "		
4...	" " " " " "	11.420 "	10.710 "	7.293 mgs.
5...	" " " " + 500 mgs. dried blood.	5.700 "		
6...	" " " " " "	Trace.*	5.700 "	2.283 "
7...	" " " " + 500 mgs. cottonseed meal.	6.670 "		
8...	" " " " " " "	2.840* "	6.670 "	3.253 "
9...	50 gms. " + 0.5 gm. "	4.000 "		
10...	" " " "	5.000 "	4.500 "	
11...	" " " " + 100 mgs. $(NH_4)_2SO_4$	16.000 "		
12...	" " " " " "	16.000 "	16.000 "	11.500 "
13...	" " " " + 500 mgs. dried blood.	5.750 "		
14...	" " " " " "	6.820 "	6.285 "	1.785 "
15...	" " " " + 500 mgs. cottonseed meal.	13.340 "		
16...	" " " " " " "	12.650 "	12.995 "	8.495 "
17...	100 gms. " + 1.0 gm. "	4.440 "		
18...	" " " "	5.000 "	4.720 "	
19...	" " " " + 50 mgs. $(NH_4)_2SO_4$	11.420 "		
20...	" " " " " "	10.000 "	10.710 "	5.990 "
21...	" " " " + 100 mgs. "	13.340 "		
22...	" " " " " "	16.000 "	14.670 "	9.950 "
23...	" " " " + 200 mgs. "	21.320 "		
24...	" " " " " "	14.000* "	21.320 "	16.600 "
25...	" " " " + 500 mgs. dried blood.	25.000 "		
26...	" " " " " "	26.670 "	25.835 "	21.115 "
27...	" " " " + 500 mgs. cottonseed meal.	32.000 "		
28...	" " " " " " "	33.340 "	32.670 "	27.950 "

The amounts of nitrate nitrogen produced from the given quantities of ammonium sulphate, dried blood and cottonseed meal were clearly affected by the volume of soil in which these materials were distributed. In the untreated soil portions 1 and 2, 9 and 10, 17 and 18 the average amounts of nitrate nitrogen found were 3.417 mgs., 4.500 mgs. and 4.720 mgs., respectively. There is shown, thus, a slight increase in the nitrate produced from the soil humus, as the quantity of soil was increased from twenty-five gms. to 100 gms. The increase was not, however, proportional to the increase in the amount of soil. Now, when the quantities of nitrate nitrogen just given are subtracted from the corresponding soil portions that had received additions of ammonium sulphate,

*Not included in the average.

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dried blood and cottonseed meal, we obtain the amounts of nitrate nitrogen produced from the materials added.

In making these calculations we find that in the twenty-five gm. portions of soil there were produced from the ammonium sulphate, dried blood and cottonseed meal, 7.293 mgs., 2.283 mgs. and 3.253 mgs., respectively, of nitrate nitrogen. In the fifty gm. portions of soil the corresponding amounts were 11.500 mgs., 1.785 mgs. and 8.495 mgs., respectively, and in the 100 gm. portions, 9.950 mgs., 21.115 mgs. and 27.950 mgs., respectively. It appears, therefore, that the maximum nitrification of the 100 mgs. ammonium sulphate was attained already in the fifty gms. portions of soil, while in the case of the dried blood and cottonseed meal the maximum nitrification was attained in the 100 gms. portions. Moreover, it is possible that with still larger quantities of soil even greater amounts of nitrate would have been produced from the dried blood and the cottonseed meal. It should be added here that in the twenty-five and fifty gm. portions of soil there was a greater tendency than there was in the 100 gm. portions toward drying out and thus retarding nitrification. This may partly account for lack of agreement in two or three of the duplicate portions, a condition that made it advisable to exclude from the averages the yields in soil portions 6 and 8.

As to the amounts of nitrate nitrogen produced from fifty, 100 and 200 mgs., respectively, of ammonium sulphate we note them to have been 5.990 mgs., 9.950 mgs. and 16.600 mgs. Increasing amounts of ammonium sulphate led, therefore, to a nearly proportional increase in the amounts of nitrate produced. However, the largest amounts of ammonium sulphate used caused, in at least one case (soil portion 24), a depression in the rate of nitrification.

The relative amounts of nitrogen transformed into nitrate from the materials used are shown in the following table:

	Ammonium Sulphate.	*Dried Blood.*	*Cottonseed Meal.*
In 25 gms. of soil.....	35.9 per cent.	4.2 per cent.	10.2 per cent.
" 50 " "	56.6 "	3.3 "	26.5 "
" 100 " "	49.0 "	39.2 "	87.3 "

The 100 mgs. of ammonium sulphate employed in this experiment contained 20.3 mgs. of nitrogen. The 500 mgs. of dried

blood and of cottonseed meal contained 53.8 mgs. and 32.0 mgs., respectively. Of these amounts there were recovered as nitrate 35.9 to 56.6 per cent. in the case of the ammonium sulphate; 3.3 to 39.2 per cent. in the case of the dried blood, and 10.2 to 87.3 per cent. in the case of the cottonseed meal. The results are instructive in showing comparatively slight nitrification of the dried blood in the smaller volumes of soil. To a lesser extent this was also true of the cottonseed meal. When, however, the weight of the soil portions were increased to 100 gms. the nitrification of both the dried blood and the cottonseed meal was intensified so as to transform 39.2 per cent. of the nitrogen of the former and 87.3 per cent. of the latter into nitrate. In case of the ammonium sulphate the varying volume of soil was not as important. Where different amounts of ammonium sulphate were used in the 100 gms. portions of soil the relative amounts changed into nitrate were:

50	mgs.	ammonium	sulphate	59.0	per cent.
100	"	"	"	49.0	"
200	"	"	"	40.9	"

The conclusions to be drawn from this experiment confirm those adduced in the ammonification experiments. It appears that in materials like cottonseed meal and dried blood the rate of decomposition is affected up to a certain point by the volume of soil in which they are distributed; that is, by variation in the physical, chemical and bacteriological factors involved. To be sure, the quantities of material employed in the present experiment were relatively very large, much greater than the amounts that are at all likely to be employed in practice. For all that, the results secured here emphasize in a practical way the importance of thorough distribution in the soil. We know that in field practice or under market-garden conditions, even when relatively small amounts of nitrogenous materials are used, the distribution may be so unsatisfactory as to bring a comparatively small amount of soil in contact with a comparatively large amount of fertilizer. Under such conditions the rate of ammonification and of nitrification is likely to be affected in an unsatisfactory manner as indicated by the present experiment.

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THE EFFECT OF ADDITIONS OF NITRATE ON THE ACCUMULATION OF NITRATES IN THE SOIL.

SERIES U.

It was observed in earlier experiments[1] that there may be a certain periodicity in the accumulation of nitrates in the soil. Where no plants are growing the amount of nitrate in the soil gradually increases up to some indefinite point, then suffers a more or less marked decrease, followed again by another period of gradual increase. The periods of gradual increase and decrease may follow one another a number of times. When leaching is excluded the periodical decrease may be largely attributed to the consumption of nitrates by bacteria and other micro-organisms which may be favored in their periodically rapid development by the very presence of large quantities of nitrates.

It is fairly safe to assume that the consumption of nitrates and their transformation into protein compounds is to be reckoned with in all nitrification experiments even though it be disguised by other factors. In view of this fact the present experiment was arranged to furnish if nothing more than an indication of the relative importance of some of the factors involved.

One hundred gram portions of fertile soil received additions of varying amounts of sodium nitrate and were then maintained at optimum moisture conditions. Determinations of nitrite and nitrate nitrogen were made in duplicate portions at the end of two, four, six and eight weeks. The arrangement of the series and the amounts of nitrite and nitrate nitrogen found are recorded in the following table:

Soil Portion No.	*Additions.*	IN 2 WEEKS.		IN 4 WEEKS.	
		Nitrate N.	*Nitrite N.*	*Nitrate N.*	*Nitrite N.*
1....	0	2.856 mgs.	.0868 mgs.	8.000 mgs.	.0608 mgs.
2....	0	2.960 "	.0812 "	7.840 "	.0868 "
3....	0.2 gms. $NaNO_3$.	42.64 "	.0768 "	32.000 "	.0940 "
4....	" "	43.06 "	.0608 "	32.000 "	.0880 "
5....	0.6 gms. "	85.32 "	.0868 "	98.400 "	.0985 "
6....	" "	85.50 "	.0868 "	100.000 "	.1012 "

[1] N. J. Sta. Rep., 1908, p. 105.

Soil Portion No.	Additions.	In 6 weeks. Nitrate N.	In 6 weeks. Nitrite N.	In 8 weeks. Nitrate N.	In 8 weeks. Nitrite N.
1....	0	8.332 mgs.	.1720 mgs.	10.000 mgs.	.2020 mgs.
2....	0	8.000 "	.2000 "	10.500 "	.2000 "
3....	0.2 gms. $NaNO_3$.	50.000 "	.3040 "	53.330 "	.1770 "
4....	" "	49.244 "	.3440 "	54.500 "	.1820 "
5....	0.6 gms. "	106.645 "	.1360 "	158.000 "	.2000 "
6....	" "	107.500 "	.1440 "	157.550 "	.2100 "

As shown by the arrangement of the experiment there were eight soil portions corresponding to each treatment. Two each of these were analyzed for nitrate and nitrite nitrogen at the end of two weeks, four weeks, six weeks and eight weeks, respectively. Subtracting 31.0 mgs. of the nitrate nitrogen added to the soil portions corresponding to numbers 3 and 4, and 93.0 mgs. of the nitrate nitrogen added to the soil portions corresponding to numbers 5 and 6, we secure the following figures:

Soil Portion No.	In 2 Weeks.	Difference.	In 4 Weeks.	Difference.
1	+ 2.856 mgs.		+ 8.000 mgs.	
2	+ 2.960 "		+ 7.840 "	
3	+ 11.640 "	+ 8.732 mgs.	+ 1.000 "	— 6.920 mgs.
4	+ 12.060 "	+ 9.152 "	+ 1.000 "	— 6.920 "
5	— 7.680 "	— 10.588 "	+ 5.400 "	— 2.520 "
6	— 7.500 "	— 10.408 "	+ 7.000 "	— 0.920 "

Soil Portion No.	In 6 Weeks.	Difference.	In 8 Weeks.	Difference.
1	+ 8.332 mgs.		+ 10.000 mgs.	
2	+ 8.000 "		+ 10.500 "	
3	+ 9.000 "	+ 0.834 mgs.	+ 22.330 "	+ 12.080 mgs.
4	+ 8.244 "	+ 0.078 "	+ 23.500 "	+ 13.750 "
5	+ 13.645 "	+ 5.479 "	+ 65.000 "	+ 54.750 "
6	+ 14.500 "	+ 6.334 "	+ 64.550 "	+ 54.300 "

The figures before us show that the addition of sodium nitrate readily affected the accumulation of nitrate nitrogen in the soil. When 0.2 gms. of $NaNO_3$ were added the accumulation of nitrate was enhanced at the end of two weeks as compared with the blank soils. In the following two weeks the accumulation of nitrate nitrogen was depressed, showing a deficit of nearly seven mgs. as compared with the corresponding blank soils. After that there was an enhanced accumulation until at the end of eight weeks the addition of the 0.2 gms. of $NaNO_3$ had resulted in a greater accu-

mulation of nitrate nitrogen equivalent, on the average, to 12.915 gms.

No less interesting are the relations in the soil portions corresponding to numbers 5 and 6. At the end of two weeks there was a deficit of more than 10.5 mgs.; at the end of four weeks the deficit was decreased to 1.720 mgs.; on the average, at the end of six weeks, there was an enhanced accumulation of nearly six mgs.; and at the end of eight weeks, of more than fifty-four mgs. In considering the data at hand it should be remembered, of course, that small amounts of nitrate may favor the decomposition of inert humus compounds. Hence, where nitrate was used in the present experiment the decay bacteria were stimulated in their growth and were able thereby to attack the organic nitrogen compounds more vigorously. At the same time a larger amount of nitrate was laid fast in the bodies of the bacteria by being converted into protein substances. Later on the bodies of the dead bacteria in their time passed through the process of ammonification and nitrification. We thus come to find that periodicity in the accumulation of nitrates in the soil may be due to both the temporary prominence of species, especially capable of transforming large amounts of nitrate into protein nitrogen, as well as to the mere rapid increase of various decay organisms, and their intense utilization of nitrates for the building of their bodies.

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VEGETATION EXPERIMENTS WITH MISCELLANEOUS MATERIALS USED AS DIRECT OR INDIRECT FERTILIZERS.

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VEGETATION EXPERIMENTS WITH MISCELLANEOUS MATERIALS USED AS DIRECT OR INDIRECT FERTILIZERS.

The Availability of the Phosphoric Acid in Phospho-Plaster.

This material is being sold by the Bowker Chemical Company and is a by-product in the manufacture of water soluble phosphate from very finely ground Florida Phosphate Rock. After treating the latter with sulphuric acid the soluble calcium phosphate and free phosphoric acid are leached out, and the residue dried in rotary furnaces. The finely divided material thus secured consists largely of gypsum and contains approximately 3.5 per cent. of total and 1.0 per cent. available phosphoric acid anhydride (P_2O_5). About 2,000 tons of phospho-plaster are produced annually by the Bowker Company, a quantity sufficiently large to render the question of its economic utilization on the farm of more or less importance.

The vegetation tests considered here were arranged with a view toward determining not so much the entire agricultural value of phospho-plaster (that would vary greatly under different conditions of soil and farm methods), but the availability of the phosphoric acid contained in it. The tests were carried out in large glass pots each containing twenty pounds of pure quartz sand. There was added to each pot,

10	gms.	$CaCO_3$.
2	"	K_2SO_4.
0.25	"	Mg. SO_4.
0.02	"	$Fe_2(SO_4)_3$.

Uniform moisture conditions were maintained in the several pots. The crop consisted of winter vetch seeded January 14th and harvested April 22d. The special treatment of the different soils and the yields of dry matter in each case are recorded in the following table:

Pot No.	Special Application.	Air-Dry Matter.	Average.	Increase.
1.....	0	1.9 gms.		
2.....	0	2.0 "	1.95 gms.	
3.....	2 gms. phospho-plaster.	4.0 "		
4.....	2 " "	4.9 "	4.45 "	2.50 gm.
5.....	6 " "	6.0 "		
6.....	6 " "	6.0 "	6.00 "	4.05 "
7.....	1 gm. acid phosphate.	7.8 "		
8.....	1 " " "	8.5 "	8.15 "	6.20 "

It will be observed that the larger amount of phospho-plaster increased the yield of dry matter to an average of six gms., while the smaller increased it to 4.45 gms. The net increase for the smaller and larger application was 2.50 gms. and 4.05 gms., respectively. In view of the fact that the acid phosphate contained about 14 per cent. of available phosphoric acid, the availability of that in the phospho-plaster is proportionately larger. The dry weights of the crop can serve only as an approximate measure of relative availability, since the crops from the different pots vary somewhat in composition. For all that, the figures before us indicate that under suitable conditions as much as 2 per cent., or even more out of the 3.5 per cent. of the phosphoric acid in phospho-plaster, may become available to the crop in a single growing season. The accompanying photograph shows the effect of the phospho-plaster and of the acid phosphate on the growth of the vetch.

When considered from the commercial and agricultural standpoint the phospho-plaster may be said to possess a value of about two dollars per ton because of the quantity and quality of the phosphoric acid contained in it, and an additional value due to the gypsum. This additional value of the 80 per cent. of gypsum that the phospho-plaster contains must be determined by local conditions. We know that when used in stables, gypsum may help to conserve a portion of the ammonia that would otherwise escape. It should be added here, however, that agricultural chemists are not unanimous in regarding gypsum of any considerable value as a conservator of ammonia. On the other hand, there is convincing evidence to the effect that gypsum improves the quality of barnyard manure by stimulating nitrification. There is no doubt, also, that from the standpoint of cleanliness and general sanitation the use of gypsum in stables is altogether desirable.

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Availability of Phosphoric Acid in Phospho-Plaster.

Furthermore, it is well known that gypsum favors nitrification in the soil as well as the growth of those bacteria that produce tubercles on the roots of clover and of other legumes. To these favorable effects of gypsum may be added its indirect action in furnishing potash to plants by reacting with the inert potash compounds in the soil.

In view of all these facts gypsum may be said to possess a certain value on dairy farms, especially those located on heavy clay soils. Obviously this value cannot be measured exactly in any particular case. It is for the individual farmer to decide in how far it is desirable for him to use gypsum in his stable to improve its sanitary condition. Should he find its use desirable from the sanitary and other standpoints, phospho-plaster should recommend itself to his consideration, for apart from the gypsum itself he will find in it two dollars' worth of phosphoric acid; in other words, he will be able to secure the gypsum itself at a comparatively low cost.

THE AVAILABILITY OF THE PHOSPHORIC ACID IN LOW-GRADE PHOSPHATE ROCK.

The low-grade rock in question is a by-product at the works of Witherbee, Sherman and Company, at Mineville, Essex county, New York. Concerning this material it was stated by the manager of the company in a letter dated October 13th, 1908: "We have here large quantities of this material, and when our works are running full would make from 150 to 250 tons per day. We are very anxious to put this material on the market as it is waste material which is removed from the iron ore by crushing and electric separation, and is what we call the 'tailings' after the magnetic portion is removed by the treatment. The material as made by us, or what we could make economically, would run from 35 to 45 per cent. B. P. or from 7 to 9 per cent. phos. (phosphorus)."

In a subsequent letter of January 11th, 1909, he wrote: "At the present time we are grinding this material with a Sturtevant emery stone mill, and the average of all of our screen tests show that 62½ per cent. will go through 100 mesh. This is much finer than the other ground raw rock phosphates, which are being sold,

but whether it is fine enough, of course, I am not the judge. My judgment is that if we are to put the material on the market, it will be necessary for us to grind the material in a tube mill so as to get it much finer, owing to its not being as high in phosphoric acid as the fine rock phosphates of Tenn. Or in other words, we would try to overcome its lower percentage of phosphoric acid by finer grinding.

"In regard to the chemical composition of the material, the daily samples from December 1st to 11th show an average of 16.25 per cent. phosphoric acid. The lowest during this time was 14.24 per cent. and the highest 17.88 per cent. From the 11th to the 30th of December we did not grind this material, but from December 30th to January 7th the average was 17.40 per cent. phosphoric acid. The lowest was 14.48 per cent. and the highest 17.99 per cent. phosphoric acid.

"During the time we shipped two carloads, and the average analysis of the samples taken from one car was 15.17 per cent. and the other 15.04 per cent. phosphoric acid. The average of five samples taken for screen tests show 15.24 per cent. phosphoric acid. We, therefore, would not care to guarantee better than 15 per cent. phosphoric acid in the material. Neither would we think it safe to guarantee, with our present means of grinding, to grind the material better than 62½ per cent. to pass 100 mesh."

The above statements were cited to show that the crude phosphate material is a by-product of considerable magnitude, amounting to many thousands of tons annually. The phosphoric acid contained in it is an asset of great value provided an economic method of utilizing it could be devised. In its present state it is not suitable for the manufacture of superphosphate, because of its comparatively low content of phosphoric acid for this purpose. This the members of the company realized, yet they believed that in a finely ground state their crude phosphate might become available in most soils. The Station was, therefore, requested to test this material in vegetation experiments, and the request was readily granted because of the possible agricultural value of the material under consideration. A twenty-five-pound sample containing 15.26 per cent. of phosphoric acid and fine enough to pass a 100 mesh screen to the extent of 68.75 per cent. was secured for this purpose.

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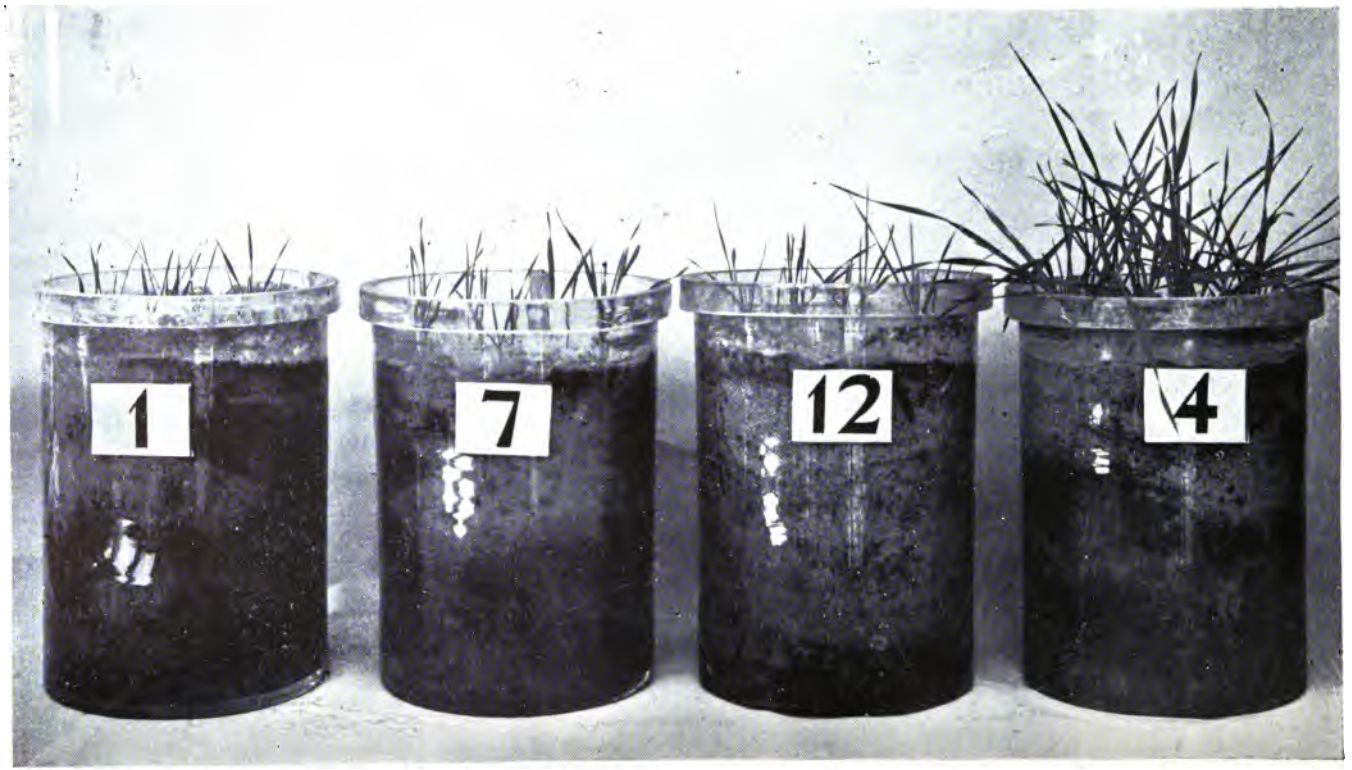

Availability of Phosphoric Acid in Low-Grade Phosphate.

The experiments were carried out in large glass pots each provided with twenty pounds of pure quartz sand containing in each case:

15 gms.	calcium carbonate.
10 "	magnesium carbonate.
3 "	Kainit.
1 gm.	muriate of potash.
2 gms.	sodium nitrate.
0.1 gm.	ferric sulphate.

The several soils portions were supplied with proper amounts of water which was thereafter maintained at a uniform proportion in all of the pots. The several soil portions were seeded with rye. The special treatment and the yields are recorded in the following table:

Soil Portion No.	*Special Treatment.*	*Yield of Dry Matter.*	*Average.*
1.....	0	0.345 gm.	
2.....	0	0.375 "	0.360 gms.
3.....	2 gms. acid phosphate.	6.000 "	
4.....	2 " " "	6.700 "	6.350 "
5.....	2 " phosphate material.	0.350 "	
6.....	2 " " "	0.475 "	0.412 "
7.....	4 " " "	0.405 "	
8.....	4 " " "	0.373 "	0.389 "
9.....	10 " " "	0.368 "	
10.....	10 " " "	0.455 "	0.411 "
11.....	20 " " "	0.315 "	
12.....	20 " " "	0.415 "	0.365 "

The yields of dry matter were small in all of the soils except those that received additions of acid phosphate. Notwithstanding the fact that the plants were starving for lack of phosphoric acid, the relatively enormous amounts of the crude phosphate material appeared incapable of meeting even partly the needs of the crop. Thus in pots 11 and 12 the applications of the phosphate material was equivalent to more than three tons per acre; nevertheless, the average yield of dry matter in these pots was 0.365 gms. as against 0.360 gms. in pots 1 and 2 that had received no addition of any phosphorus carrying material except that in the seed. On the other hand, the application of two gms. of acid phosphate increased the yield to 6.350 gms. of dry matter. These relations are brought out graphically in the accompanying photograph, showing the growth in pots 1, 7, 12 and 4.

Making due allowance for the fact that the soil used in these experiments was devoid of humus, it still remains true that the relatively enormous quantities of phosphatic material employed should have furnished at least some available phosphorous to the plants. That such was not the case was already shown. Hardly anything else could have been expected in view of the very extensive experiments abroad and in this country showing the very inert character of ground rock phosphate in all excepting acid soils. Hence it appears that if the material in question is to be exploited for agricultural uses in a profitable and effective manner it will have to be mixed with high-grade phosphate rock and acidulated so as to furnish 12 to 14 per cent. of available phosphoric acid. It is possible, also, that where large amounts of waste sulphuric acid are available this material could be used for the manufacture of free phosphoric acid or of monocalcic phosphate to be employed in the making of superphosphate or in other industrial processes.

EXPERIMENTS ON THE AVAILABILITY OF NITROGEN IN PEAT.

The various deposits of peat scattered throughout the State, and particularly throughout Northern New Jersey, possess, like the deposits in other States, a potential value as a source of fuel and of plant-food. The deposits in Bergen, Morris, Sussex and Warren counties are especially notable in this respect and instances of their attempted utilization are fairly numerous. Dr. George H. Cook stated as long ago as 1866[1] that peat was "long used in Chatham township, Morris county, and to a smaller extent in many other places." The peat was here used as fuel, as it was also at Belleville, Essex county, and at Allendale, Bergen county. But as a fuel, pure and simple, peat could not successfully compete with bituminous and anthracite coal and the different plants established for this purpose were doomed to stagnation and failure.

More recently the utilization of certain peat deposits in New Jersey has been attempted from a different standpoint. Portions of the extensive deposits in Warren county, known as the Great Meadows, or the Pequest Meadows, were purchased by companies

[1] New Jersey Geological Survey, 1886, p. 13.

Fig. 1.

the soil flying out from the paddle-wheel. The ground at the side of the machine having been previously leveled, this thinly spread layer when dry is scraped into heaps by a motor-scraper * * *. (Fig. 3.) After the dry soil has been scraped into heaps, it is loaded onto dump cars by the electric loader. * * * As the cars are filled, they are run to the factory * * * and dumped on the pile * * *. (Fig. 4.) This sun-dried material may now be bagged and shipped without further treatment, and part of it is so disposed of."

Inquiries concerning the agricultural value of Alphano or Humus, as it was sometimes called, began to come to the Station in the fall of 1908. The replies to these inquiries were based on the general experience with peat elsewhere and on the composition of samples derived from the Great Meadows and similar deposits. For instance, a sample collected by Parmelee at the east side of the swamp, three miles north of Danville and 200 yards west of the railroad, on the property of the Alphano Company, had the following composition:[1]

Moisture	20.84 %
Ash	11.04 %
Nitrogen	1.98 %

A sample drawn from the premises of the American Agricultural Chemical Company contained 1.96 per cent. of nitrogen and 13.31 per cent. of ash, and one drawn on the premises of the Woodbridge Manufacturing Company contained 2.45 per cent. of nitrogen and 8.62 per cent. of ash. It appeared, therefore, that, from the fertilizer standpoint, the chief value of the humus lay in its nitrogen, since there was not much to be expected from the phosphoric acid, potash and lime contained in it, as indicated by earlier analyses of samples from similar deposits. For instance, Cook reported[2] the following analyses of air-dry samples, from the Black Brook Meadows:

[1] 1 c. p. 305.
[2] Geology of New Jersey, 1868, 481, 700.

Fig. 3.

Fig. 4.

	I.	II.
Organic matter	65.61	66.87
Water	16.16	15.15
Oxides of iron and alumina	3.19	3.97
Lime	3.86	3.17
Magnesia	0.37	0.39
Potash	0.31	0.27
Phosphoric acid	0.93	0.10
Sulphuric acid	0.89	2.46
Carbonic acid	0.09	....
Silica	8.64	7.63

To these may be added the analysis of a sample submitted by the Alphano Company to Stilwell and Gladding. It contained:

Organic matter	84.10
Phosphoric acid	0.49
Potash	0.05
Lime	8.36
Silica	7.00

Among the several mineral constituents recorded above, the potash can scarcely be considered as possessing any but a very slight value, because of its inert character. As to the phosphoric acid an average content of 0.5 per cent. would add to each ton of humus a value of 50 cents, provided all of the phosphoric acid were readily available. Such is far from being the case; hence a value of 25 to 30 cents per ton would be a generous one to add to that of one ton of the air-dry material.

It is evident thus that the nitrogen of the humus is its most valuable plant-food constituent. Taking the average proportion of nitrogen present in the air-dry humus at 2 per cent. we have a total content per ton of forty pounds. Now, if this were all readily available the humus would be worth $5 per ton for its nitrogen alone. Unfortunately the nitrogen compounds of peat are very inert and when applied directly to the soil do not become available readily. For all that it was deemed just to all parties concerned to institute a series of vegetation tests on the relative availability of the nitrogen in Alphano. Through the kindness of Mr. John N. Hoff of the Alphano Company, two samples were secured for this purpose, one designated as *sun-dry* and containing 1.36 per cent of nitrogen; the other designated as *bone-dry* (kiln-dried) and containing 2.66 per cent. of nitrogen.

The vegetation experiment was carried out in tarred glass pots containing in each case twenty pounds of pure quartz sand. The following materials were added to each soil:

20	gms.	ground oyster shell lime.
4	"	acid phosphate.
2	"	muriate of potash.
0.5	"	magnesium sulphate.
0.05	"	ferric sulphate.

The proper amount of moisture was supplied in each case, and optimum moisture conditions were maintained as far as possible throughout the experiment. Rye was the crop grown, the same number of seeds having been planted in each soil. The special treatment and the yields of both dry matter and of nitrogen are recorded in the following table:

Pot No.	*Special Treatment.*	*Dry Matter. gms.*	*Nitrogen. %*	*Nitrogen. mgs.*	*Aver. mgs.*
1..... 0		1.8	1.113	20.03	20.92
2..... 0		2.5	0.873	21.82	
3..... 1	gm. N in sun-dry humus.	7.6	1.025	77.90	
4..... 2	" " " "	11.4	1.470	164.34	
5..... 3	" " " "	13.5	1.597	215.59	
6..... 1	" " bone-dry humus.	6.5	0.883	57.39	
7..... 2	" " " "	9.5	1.156	109.82	
8..... 3	" " " "	11.2	1.573	176.18	

Examining the results secured we find that the increasing amounts of humus led to increasing yields of dry matter. Thus the application of one gm. of nitrogen in the form of sun-dry humus increased the yield of dry matter from 2.15 gms. (average of pots 1 and 2) to 7.6 gms., while two gms. of humus nitrogen increased it to 11.4 gms., and three gms. to 13.5 gms. of dry matter. A similar increase occurred also in the pots where bone-dry humus was used, except that the absolute yields were not as large. Furthermore, the increasing amounts of humus increased the proportionate amount of nitrogen in the dry matter. The per cent. of nitrogen in the dry matter from the untreated soils was, on the average, 0.993. The percentage of nitrogen in the dry matter from pots 3, 4 and 5 were 1.025, 1.470 and 1.597, respectively. Similar relations were found in the dry matter of the pots where bone-dry humus was applied. The larger applications of humus thus led to the production of more dry matter, of dry matter relatively

Fig. 5.

richer in nitrogen, and of absolutely greater quantities of nitrogen in the crop. The average yield of nitrogen from pots 1 and 2 was 20.92 mgs., while from pots 3, 4 and 5 they were 77.90 mgs., 164.34 mgs. and 215.59 mgs., respectively. The effect of the increasing amounts of humus nitrogen is shown graphically in the accompanying photograph. (Fig. 5.)

Subtracting the yields of nitrogen of the untreated pots from those in the treated pots, and calculating the results on the basis of the amounts applied, we find the following proportions of humus nitrogen recovered in each instance:

Pot No.	*Proportion of the Applied Nitrogen Recovered.*
1	.....
2	.....
3	5.69 per cent.
4	7.17 "
5	6.49 "
6	3.65 "
7	4.44 "
8	5.17 "

We observe, in the first place, that the nitrogen of the sun-dry humus was more available than that of the bone-dry humus. Evidently the drying of the material at higher temperatures reduced still further its resistance to the decomposition processes in the soil. At best, however, the availability of the humus nitrogen was very low. The highest recovery of the applied nitrogen was only 7.17 per cent. in the case of the sun-dry humus, and only 5.17 per cent. in the case of the bone-dry humus. Now, in the experiments carried on at this Station[1] and elsewhere[2] it has been shown that even nitrate, the most available of the nitrogen compounds, is not completely recovered in the crop. Under field conditions only sixty-five to seventy pounds out of every 100 pounds applied are recovered under average conditions. The proportionate amounts recovered from ammonium salts or from organic nitrogenous fertilizers are still lower. It is usual to employ nitrate nitrogen as the standard against which the relative availability of other forms of nitrogen is measured. The relative availability of nitrate nitrogen is taken as 100 and the others are graded accordingly. By employing this standard of measurement we find, therefore, that the relative

[1] Bull. 221, N. J. Exp. Station.

[2] Arbeiten der Deutschen Landwirtschafts-Gesellschaft. Heft 129, Berlin, 1907.

availability of the nitrogen in sun-dry humus is about ten. In other words, for the purposes of crop-growth a pound of humus nitrogen is worth one-tenth as much as a pound of nitrate nitrogen. Taking the retail value of nitrate nitrogen at twenty cents per pound we must assign to the humus nitrogen a value of two cents per pound.

With these figures as a basis it remains now to calculate the value of Alphano, or humus (used in this special sense). It will be remembered that the sun-dried humus employed in the present experiments contained 1.346 per cent. of nitrogen; or, in round numbers, twenty-seven pounds per ton. This figure is probably below the average for sun-dried humus; it should be about 1.5 per cent. or thirty pounds of nitrogen per ton. At two cents per pound the nitrogen in a ton of humus should be worth 60 cents. In air-dry humus containing 2.0 per cent. or forty pounds of nitrogen per ton the value would be increased to 80 cents per ton. Making a further allowance for the phosphorus contained in the humus the value of the material might be increased to $1 per ton, on the whole, a generous allowance.

"But," it will be asked, "does not the humus furnish organic matter? And does that possess no value at all?" To this it may be answered that the humus does supply organic matter which may comprise two-thirds or three-quarters of the total weight of the air-dry material. Nevertheless, this organic matter, like the nitrogen contained in it is inert. In the process of its formation the readily decomposable portions gradually vanished. The residue persisted because of its very inertness. But we know that organic matter is useful in cultivated soil not merely because it improves the water-holding power and the aeration of the latter, but also because it serves as food to the vast numbers of soil bacteria that are indispensable for the production of sufficient amounts of available nitrogen compounds, and useful for the production of sufficient amounts of available phosphoric acid, potash and lime. Measured by the latter standard the humus is not a satisfactory form of organic matter. It is too old, too inert, and therefore entirely too expensive for this purpose. Hence, it may be reiterated here that Alphano in the sun-dry or air-dry condition should not cost more than $1 per ton, a price at which its commercial exploitation could not be made profitable.

It is to be hoped, however, that other means may be found for utilizing these large deposits profitably and with benefit to agricul-

tural interests. For one thing, it might be used as litter in poultry establishments, where it would add much to the sanitary conditions of the hen-houses, and would, in addition, help to conserve a portion of the ammonia that ordinarily escapes into the air. Similarly it could be employed in stables, barns and cattle stalls, where it would help to conserve the ammonia and add to the comfort of the animals. Moreover, the mingling of the inert nitrogen compounds of the humus with the readily decomposable nitrogen compounds of the liquid manure of cattle or of the readily fermentable droppings of fowls would stimulate the decomposition of the inert substances, and would render their nitrogen much more available. The relative availability might be increased to thirty, forty or fifty, thus multiplying the value of the nitrogen by as many units. It needs no complicated calculation to show that by increasing, in this manner, the value of the peat nitrogen from two cents to ten cents per pound, the value per ton of material would be proportionately increased from $1 to $5, a price at which the exploitation of the peat deposits should become highly profitable.

There is no objection to the use of peat as a filler in complete fertilizers or as a diluent in incomplete fertilizers, like dried blood, provided that no attempt is made to charge for the peat nitrogen the same price that is charged for dried blood nitrogen. Unfortunately this is exactly what is being done in many instances. It requires no great business acumen to realize that 800 pounds of dried blood containing 12.5 per cent. of nitrogen may be mixed with 200 pounds of dry peat containing 2.5 per cent. of nitrogen. The resulting mixture will contain 10.5 per cent. of nitrogen that may be sold at retail for twenty to twenty-two cents per pound. In other words, 200 pounds of peat may thus be sold for $1, or at the rate of $10 per ton. It need scarcely be pointed out here that business morality should condemn the sale of nitrogen compounds with a relative availability of ten at the same price charged for nitrogen compounds with a relative availability of sixty or seventy. In so far as the experiment stations are concerned the detection of materials of a low rate of availability is not a matter of great difficulty. Chemical as well as bacteriological methods may be employed for this purpose, and a method of classification may be established that would differentiate with reasonable accuracy and equity nitrogen compounds of varying degrees of availability.

EXPERIMENTS ON THE AGRICULTURAL VALUE OF CALCIUM CARBONATE CONTAINING A SMALL PROPORTION OF BORATES.

Carbonate of lime is one of the by-products at the works of the Pacific Coast Borax Company at Bayonne. The estimated production of this material is about twenty thousand tons annually, a quantity sufficiently large to make it a possibly important factor in the supplying of agricultural lime to the farmers of New Jersey.

This carbonate of lime as it leaves the works is in a finely divided state in so far as the individual particles are concerned, yet the entire mass is pasty and wet and as such not suitable for disposal to farmers. Formerly the company made no attempt to prepare a salable article out of this lime. It was loaded in scows and dumped in the bay. At the suggestion of George A. Bush, to whom the writer is indebted for samples of this lime, the material is dried, ground to a fine powder and sold for agricultural uses. Following are the analyses, made at the factory, of two distinct samples of the carbonate. In the second sample the weights of iron and alumina are combined:

	I.		II.	
Silica	8.67	per cent.	9.71	per cent.
Alumina	1.81	"	1.86	"
Oxide of iron	.35	"		
Lime	45.84	"	44.56	"
Magnesia	2.75	"	3.65	"
Boric acid (anhydrous)	2.36	"	2.00	"
Carbon dioxide	33.85	"	33.04	"
Sodium oxide	1.16	"	1.76	"
Water	2.02	"	2.36	"

Small amounts of chlorides, sulphates and organic matter.

Similar analyses of other samples show that the material contains, on the average, 75 to 80 per cent. of calcium and magnesium carbonates. In consideration of the fact, therefore, that the material is very fine and that it may be readily and uniformly distributed by means of a fertilizer distributor, the price at which it is sold, namely, $2 per ton, is a very reasonable one. In fact, in so far as the selling price and ease of application are concerned this is one of the cheapest limes in Middle Jersey. Moreover, it contains an appreciable amount of soda which may help to make

available inert potash compounds in the soil. But there is still another constituent present in this lime that should be considered as possibly detracting from its value. It will be noticed that it contains more than 2 per cent. of anhydrous boric acid, a material that may prove detrimental to soil bacteria and to the plants themselves when it is present in sufficiently large amounts. Since the lime was being placed on the market and prices were being quoted to prospective buyers, a number of inquiries reached the Station concerning the agricultural value of this by-product. It became desirable, therefore, to carry out some experiments with this lime, particularly as to the effect of the boric acid present in it on the growth of plants. Accordingly, a series of vegetation tests was instituted in large earthenware pots, each containing twenty pounds of fertile clay loam soil. Each soil portion received an addition of four gms. of acid phosphate and two gms. of muriate of potash. The crop used in this experiment was winter vetch. The special lime treatment accorded to each soil portion, and the yields of dry matter are recorded in the following table:

Pot No.	*Lime Applied.*	*Yield of Dry Matter.*
1.....	0	21.2 gms.
2.....	4 gms. burned oyster shell lime,	20.6 "
3.....	8 " " " " "	23.2 "
4.....	16 " " " " "	21.0 "
5.....	0	15.0 "
6.....	4 " carbonate from Bayonne,	18.0 "
7.....	8 " " " "	18.2 "
8.....	16 " " " "	16.2 "

The vetch germinated satisfactorily in all of the pots and made good growth in the following weeks. In the course of time it was observed that the tips of the leaves of the vetch plants in pots 7 and 8 shrivelled and dried up. Otherwise the plants appeared to be healthy. The yields of dry matter indicate, at first sight, that the carbonate from Bayonne depressed plant development, since the equivalent applications of oyster shell lime produced larger yields. It will be observed, at the same time, that the lowest yield of dry matter was secured in pot 5 where the soil received no application of lime whatsoever. It appears thus that the lower yields in pots 5 to 8 as compared with the yields in the corresponding pots 1 to 4 were due to other causes partly or wholly. For one thing, the position of the pots on the bench and the unequal

amount of shade which they received could not always be equalized by moving the pots from time to time. Hence it seems that applications of this lime, equivalent to one ton, or even one and one-half tons, per acre need not in any way reduce the yields of legumes and probably also of non-legumes on account of the boric acid present in it. It is possible that even larger applications would not prove injurious; nevertheless it would be safer not to use amounts larger than those indicated, especially on open sandy soil and in connection with crops like vetch and crimson clover. But within the limits given the amounts are sufficiently large for all practical purposes. Further tests are to be made to determine whether the cumulative effect of the boric acid is at all to be feared; in other words, whether applications of say one ton to the acre, repeated every three or four years, may not in time produce undesirable results. For the present, however, one need scarcely hesitate to use this lime for single applications, and its low price and superior mechanical conditions should recommend its application to land in need of lime.[1]

EXPERIMENTS ON THE EFFECT OF GYPSUM CONTAINING BORIC ACID ON THE GROWTH OF RYE.

Aside from the calcium carbonate just considered, the by-products at the works of the Pacific Coast Borax Company include also gypsum. This material, like the carbonate, leaves the works in a wet condition, but is dried, ground, and in part, passed through bolting cloth. The bolted material sells at $4 per ton, the rest at $3. The entire annual production of the gypsum is estimated at 5,000 tons, a very considerable quantity of material. Following is the analysis, made at the works, of a sample of the gypsum:

Lime	37.28	per cent.
Sulphuric acid anhydride (SO_3)	51.40	" "
Silica	4.48	" "
Alumina and iron oxide	1.59	" "
Boric acid (anhydrous)	2.10	" "
Magnesia	.24	" "
Water	2.91	" "

[1] Additional experiments made since the above was written make it appear that the injurious effect of the boric acid is greater than was at first indicated. It would be best not to use this lime at all.

The important constituents, from the agricultural standpoint, are the calcium sulphate and the boric acid. The sulphate according to the analysis just given, is present to the extent of over 88.5 per cent., and the boric acid anhydride to the extent of more than two per cent. Some of the samples probably contain nearly three per cent. of boric acid anhydride, equivalent to more than five per cent. of boric acid.

On the farm gypsum may possess a certain value for use in the stables and barn. As we already noted in connection with phosphoplaster, gypsum may be employed for improving the sanitary conditions of the stable, for conserving some of the volatile nitrogen compounds, and for stimulating certain bacteriological activities in the manure heap. Now, since boric acid is an antiseptic the question naturally arose whether it might not favor the conservation of ammonia in manure by retarding the development of the bacteria. On the other hand, this retarding action, while desirable in the stable from the sanitary standpoint, and in the manure heap from the standpoint of conserving ammonia, might prove objectionable in the soil by diminishing the supply to the crop of available plant-food.

In order to determine whether such retarding action would really occur in the soil it was decided to employ the gypsum in a series of greenhouse tests. The experiment was carried out in large glass pots, each containing twenty pounds of fertile, clay-loam soil. As a check on the action of the gypsum from Bayonne another sample of gypsum containing no boric acid was used in the same manner in a number of other pots. An attempt was made, as in the preceding experiments, to maintain uniform moisture conditions. The crop grown in this series was rye, the same number of kernels having been planted in each pot. The following table contains a record of the amounts of the two kinds of gypsum used, and also of the dry weights of rye secured from each pot:

Pot No.	*Application.*	*Yield of Dry Matter.*	*Average.*
1	0	9.2 gms.	
2	0	9.5 "	9.35 gms.
3	3 gms. gypsum,	9.5 "	
4	" "	9.0 "	9.25 "
5	6 gms. "	9.3 "	
6	" "	10.0 "	9.65 "
7	3 gms. gypsum with boric acid,	9.7 "	
8	" " " " "	9.7 "	9.70 "
9	6 gms. " " " "	10.1 "	
10	" " " " "	10.1 "	10.10 "

We note that the yields were quite uniform. The ordinary gypsum showed, perhaps, a tendency to increase the yields in pots 5 and 6, where the larger applications were made. Similarly, the gypsum with the boric acid appeared to favor slightly the growth of rye. At any rate, the average yield of dry matter was 9.70 gms. in pots 7 and 8, and 10.10 gms. in pots 9 and 10, whereas in the corresponding soils, where ordinary gypsum was employed, the yields of dry matter were 9.25 gms. and 9.65 gms., respectively. It appears thus that the boric acid present in the gypsum did not depress the growth of the rye even though used in relatively large amounts. For instance, in pots 5 and 6, and 9 and 10, the gypsum was used at the rate of nearly one ton per acre, an amount that is not likely to be employed in practice when the gypsum is used as an absorbent in the stables, and applied to the land only when mixed with manure. It may be concluded, therefore, that the gypsum from Bayonne may be employed in stables and barns, when sanitary reasons may make this desirable, without fear of producing undesirable effects in the soil.

GREENSAND MARL AS A SOURCE OF PLANT-FOOD IN SANDY SOILS.

Greensand marl has played a conspicuous role in the development of certain sections of New Jersey. But for the persistent use of marl throughout the first half of the nineteenth century, large areas now highly productive would raise much smaller crops. "The marl which has been described in the preceding pages," wrote Cook in 1868, "has been of incalculable value to the country in which it is found.[1] It has raised it from the lowest stage of agricultural exhaustion to a high state of improvement. Found in places where no capital and but little labor were needed to get it, the poorest have been able to avail themselves of its benefits. Lands which, in the old style of cultivation, had to lie fallow, by the use of marl produce heavy crops of clover, and grow rich while resting. Thousands of acres of land, which had been worn out and left in commons, are now, by the use of this fertilizer, yielding crops of the finest quality. Instances are pointed out everywhere in the marl district of farms which in former times would not support a

[1] Geology of New Jersey, Newark, 1868, p. 442.

family, but are now making their owners rich from their productiveness. Bare sands, by the application of marl, are made to grow clover, and then crops of corn, potatoes and wheat. What are supposed to be pine barrens by the use of marl are made into fruitful land." Quoting from Gordon's History and Gazetteer of New Jersey, published in 1830, he adds that: "It would be difficult to calculate the advantages which the State has gained and will yet derive from the use of marl. It has already saved some districts from depopulation, and increased the inhabitants of others, and may, one day, contribute to convert the sandy and pine deserts into regions of agricultural wealth."

This enthusiastic estimate of greensand marl as a source of soil fertility had, at that time, ample justification in the results secured from its use. The earliest applications of marl were made in Monmouth county soon after the beginning of the nineteenth century, although it was undoubtedly employed in isolated instances, and, on a small scale, for some years before that. The use of marl gradually extended until the amount spread aggregated one million tons per annum, as estimated by Cook in 1868. Most of this was due to local consumption, since many farmers in the marl area opened pits of their own. Aside from this, however, large quantities of marl were shipped to points more or less distant by rail. For instance, in 1867, the following amounts were shipped by several companies:

Squankum Marl Company	20,000 tons.
Freehold and Jamesburg Agricultural R. R.	18,340 "
Pemberton Marl Company	25,000 "
Camden and Atlantic R. R.	11,000 "
West Jersey Marl Company	52,000 "

In the early seventies the use of marl probably attained its maximum. Farmers in the marl area or within a few miles of it regarded it as a duty to apply a certain amount of marl every fall and winter. But the use of marl was extended also to sections of the State more or less distant from the marl beds. Scarcely a decade later the quantity of marl used had decreased to a very marked extent. The phosphate rock from the southern States, the potash salts from the German mines, and the nitrate of soda from Chili made serious inroads into the marl trade until in the last

decade of the nineteenth century but little remained of its former importance.

In looking for the causes of the popularity and wide renown of marl and for the reasons of its rapid decline in popular favor we find that it served its purpose well in the early days of intensive farming. It was then a source of phosphoric acid and of potash, and it brought back to depleted soils a vigorous growth of clover. It was thus that nitrogen, phosphoric acid and potash were restored to impoverished land. Fifty or sixty years of marling were bound to produce their effect, thanks not only to the thousands of pounds of plant-food actually added to the soil, but also to the improved mechanical condition. Yet even in the days of cheap labor the burden of marling proved a severe economic drain on the resources of farming communities. This is well illustrated, for instance, by the schedule of prices for marl dug by the Pemberton Marl Company at Birmingham. The company undertook to deliver marl at the following rates per ton: Birmingham, 80 cents; Mount Holly, 95 cents; Burlington, $1.25; Camden, $1.70; Bordentown, $1.90; South Amboy, $3.00; Trenton, $2.10; New Brunswick, $2.90; Flemington, $2.95; and Belvidere, $3.95. We see from this schedule that marl was shipped not only for considerable distances, but that the prices paid for it were hardly justified by the amounts of plant-food which it furnished.

The analyses of samples of this marl show that their composition was variable. The content of phosphoric acid, their most valuable constituent, was frequently less than 1.5 per cent., and seldom more than 2.0 per cent. Their content of potash was as high as 5 or 6 per cent., and frequently even higher. In view of the fact that water-soluble phosphoric acid in superphosphate can now be bought for five cents or less per pound, and that potash as muriate or sulphate can be bought as cheaply, these constituents in marl must possess a smaller value on account of their slighter availability. Everything considered, the marl in question can scarcely be allowed a value greater than $1 per ton; and even the Squankum marls, the best of their kind, cannot be worth more than $1.50 per ton as a source of plant-food.

Apart from its possible value as a source of plant-food marl may exert a marked effect on the physical properties of certain soils. To be sure no definite value can be placed on this function of marl,

yet it is not unreasonable to expect that when it is applied in large quantities it may render a valuable service in this direction. It is not improbable, at any rate, that notwithstanding the newer and cheaper sources of phosphoric acid the marls of New Jersey may yet regain a part of their former significance within the marl area itself. At points further removed from the marl beds it cannot under the most favorable conditions compete with superphosphate and potash salts.

An opportunity to test the value of the marl as a source of plant-food and perhaps also as an ameliorant of the physical conditions of the soil was offered to the writer in the spring of 1908. Mr. J. A. Haskell, who had recently purchased a farm at Red Bank, discovered on his land an old marl pit from which thousands of loads of marl had been removed at one time. A sample of marl analysed in our laboratory was found to contain 0.58 per cent. of phosphoric acid and 4.14 per cent. of potash. The marl in question was, therefore, of inferior quality, since it was poor in phosphoric acid. However, it was expected to make up for the small proportion of phosphoric acid by the application of large quantities of the material. Accordingly, one acre of land was carefully measured off and staked out into twenty equal plots. The treatment of the several plots was as follows:

Plot No.	*Treatment.*
1	Nothing.
2	Marl.
3	Nitrogen.
4	Phosphoric acid.
5	Potash.
6	Nitrogen and phosphoric acid.
7	Nitrogen and potash.
8	Phosphoric acid and potash.
9	Nitrogen, phosphoric acid and potash.
10	Nothing.
11	Marl.
12	Marl and nitrogen.
13	Marl and phosphoric acid.
14	Marl and potash.
15	Marl, nitrogen and phosphoric acid.
16	Marl, nitrogen and potash.
17	Marl, phosphoric acid and potash.
18	Marl, nitrogen, phosphoric acid and potash.
19	Marl.
20	Marl.

On plots 2 and 11 to 18 the marl was applied at the rate of twenty tons per acre; on plot 19 at the rate of ten tons per acre, and on plot twenty at the rate of thirty tons per acre. The nitrogen was applied as nitrate of soda and dried blood at the rate of 200 pounds for each. Phosphoric acid was applied as acid phosphate at the rate of 600 pounds per acre, and the potash as muriate at the rate of 100 pounds per acre. Corn was then planted on all of the twenty plots. Previous to the application of the fertilizers and marl the entire area received a dressing of lime at the rate of 1,500 pounds per acre. It will be seen from the plan that the applications were arranged so as to show the influence of the marl when used either alone or in combination. Any influence of the marl on the texture of the soil should have been shown at least on the plots where the application was at the rate of thirty tons per acre.

The corn was harvested in September, and the weights of the ears and stalks recorded separately. The weights found were:

	Ears.		*Stalks.*	
	Per Plot.	*Per Acre.*	*Per Plot.*	*Per Acre.*
Plot No.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1	120.0	2,400	235	4,900
2	125.0	2,500	235	4,700
3	220.0	4,400	255	5,100
4	96.5	1.930	220	4,400
5	165.0	3,300	280	5,600
6	222.5	4,450	290	5,400
7	205.0	4.100	290	5.800
8	149.5	2,990	240	4,800
9	210.0	4,020	295	5,900
10	100.0	2,000	195	3.900
11	95.0	1,900	265	5,300
12	180.0	3,600	255	5,100
13	66.5	1,330	215	4,300
14	103.5	2,070	215	4.300
15	168.5	3,370	255	5,100
16	169.0	3,380	240	4,800
17	142.0	2.840	240	4.800
18	112.0	2,240	175	3.500
19	110.0	2,200	185	3,700
20	105.0	2.100	200	4.000

INCREASE OVER THE CHECK PLOTS PER ACRE.

Plot No.	Ears Lbs.	Stalks Lbs.	Total Lbs.	Plot No.	Ears Lbs.	Stalks Lbs.	Total Lbs.
1	.....	.....	.....	11	*300	1,000	700
2	300	400	700	12	1,400	800	2.200
3	2,200	800	3,000	13	*870	.....	*870
4	*270	100	*170	14	*130	.....	*130
5	1,100	1.300	2,400	15	1,170	800	1,970
6	2,300	1,500	3,730	16	1,180	500	1,680
7	1,900	1,500	3,400	17	640	500	1,140
8	790	500	1,290	18	40	*800	*760
9	1,820	1,600	3,420	19	.....	*600	*600
10	.....	.....	.....	20	*100	*300	*400

Taking the yields of plot 1 and of plots 3 to 10 on which no marl was applied, we find the largest increase over the average yields on the check plots 1 and 10 on plots 3, 6, 7 and 9. In each instance an application of nitrogen was made. Evidently, therefore, the soil was particularly deficient in nitrogen. On plot 4 where phosphoric acid was applied there was a slight decrease as compared with the average yields on the check plots. This could be explained, perhaps, by the assumption that the phosphoric acid encouraged the growth of soil organisms which in turn used up some of the available nitrogen in the soil. On plot 5 where potash alone was applied there was a very appreciable increase, namely, 1,100 pounds of ears and 1,300 pounds of stalks per acre.

On plot 6 where nitrogen and phosphoric acid were used together the increase in yield was greater than on plot 3 where nitrogen alone was applied. In the presence of a larger supply of nitrogen the phosphoric acid evidently failed to exert a depressing effect. On plot 7 where nitrogen and potash were used together, the increase was also greater than that on plot 3, but less than that on plot 6. On plot 8 where potash and phosphoric acid were used together, the increase was smaller than that on plot 5 where potash alone was used. We note again, therefore, that without the nitrogen the phosphoric acid exerted a depressing effect. On plot 9 where nitrogen, phosphoric acid and potash were used together, the increase was again greater than that on plot 3, but smaller than that on plot 6.

Comparing now the foregoing results with the corresponding yields on plots 11 to 20 where marl was employed together with one or more of the fertilizer constituents, we find again that the

*Decrease.

largest yields were secured on plots 12, 15 and 16. Also here nitrogen was applied in each instance. The only remaining plot to which nitrogen was applied, namely, plot 18, failed to give an increase largely on account of injury to a number of plants during the growing season. Plot 13 where marl and phosphoric acid were used showed a very marked decrease as compared with the check plots. A decrease occurred also on plot 14 where potash and marl were employed, although the decrease was slighter here than on the preceding plot. Plot 17, which received marl, phosphoric acid and potash, showed an increase like the corresponding plot 8 where the marl was left out.

As to the influence of the marl itself the results of its application were evidently negative. Where used alone it gave a decrease in two out of four plots, and but a slight increase in the other two. When used in conjunction with one or more of the fertilizer constituents, it led to yields smaller than those on the corresponding plots where the marl was left out. Thus the yield of plot 12 was smaller than that of plot 3; the yield of plot 13 smaller than that of plot 4, &c.

Now, since the controlling element in the soil of the several plots was nitrogen, it could not be expected that the marl would by itself lead to a crop increase. If at all, it could do that only indirectly by creating more favorable moisture conditions for the decomposition of the humus and the formation of available nitrogen compounds. Furthermore, it could, perhaps, favor the growth of non-symbiotic nitrogen-fixing bacteria and make possible thereby a better supply of nitrogen to the crop. Besides, if a considerable portion of the potash in the marl were available a crop increase should have resulted for the same reason that muriate of potash on plot 5 produced a marked increase. We see, however, that the marl had failed to promote the growth of the corn either directly or indirectly. Judged by the returns of a single season and a single crop the value of the marl is a purely negative one, and its use on the land would hardly be justified even when the cost of hauling and spreading is slight. At the same time it would be hardly proper to regard the experiments recorded above as conclusive for all conditions. In other seasons and with other crops, particularly legumes, the results may be different. It does not seem probable, at any rate, that the enormous amounts of potash and the very large quantities of phosphoric acid added to the soil

in twenty or thirty tons of marl would permanently remain of no value. With 4 per cent. of potash and 0.5 per cent. of phosphoric acid, twenty tons of marl would add to the soil 1,600 pounds and 200 pounds, respectively, of these constituents. It only needs the presence of decomposing organic matter, of a vigorous bacterial flora, and of a generous supply of nitrogen compounds to change these inert materials into available plant-food. Herein lies the significance of past operations in the marl area and the zone adjacent to it, since many hundreds of tons of marl added to every acre of land in the course of decades thousands of pounds of potash and of phosphoric acid. Where systems of green manuring have found intelligent application on these soils, the wheat, crimson clover and cow peas returned to the land have forced a large portion of these inert constituents into active circulation. It is thus that in sections of Monmouth county the crop yields on many acres are constantly increasing even though not a pound of animal manure has been applied to them in years. When regarded in this light, the vast capital accumulated by past applications of marl still awaits a profitable investment. Whatever the economic significance of future marling, the patient digging, hauling and spreading of years long passed still await their richest reward.

Aside from the plot experiments described above, vegetation experiment was carried out at the Station in order to test the availability of the potash in marl in the presence of gypsum and of carbonate of lime. The experiment embraced thirty-two portions of white quartz sand each weighing forty-five pounds. The sand was placed in tared boxes made of galvanized sheet iron. The fertilizer materials added to each portion of sand consisted of

5	gms.	acid phosphate.
2	"	calcium nitrate.
0.5	"	magnesium sulphate.
0.05	"	ferric sulphate.

These materials were applied uniformly in all of the boxes. In addition to these there were employed varying amounts of marl, gypsum, carbonate of lime, and sulphate of potash, as is shown in the following table. The moisture content of the sand was maintained at 12 per cent. during the vegetation period by the addition of water at more or less regular intervals. The millet seeded in the boxes germinated satisfactorily, but later on the stand proved

to be somewhat uneven. The crop was harvested after the seed was well advanced toward maturity, and was then dried and weighed. The weights of the dry matter, and the special treatment accorded to the different portions of sand, are recorded in the following table:

Box No.	*Special Treatment.*	*Weight of Dry Matter.*	*Average.*
1	0	17 gms.	
2	0	15 "	16 gms.
3	10 gms. marl.	14 "	
4	" "	16 "	15 "
5	20 gms. "	16 "	
6	" "	12 "	14 "
7	50 gms. "	14 "	
8	" "	15 "	14.5 "
9	10 gms. " + 2.5 gms. $CaSO_4$.	15 "	
10	" " " "	14 "	14.5 "
11	" " + 5.0 gms. "	16 "	
12	" " " "	14 "	15 "
13	20 gms. " + 2.5 gms. "	14 "	
14	" " " "	13 "	13.5 "
15	" " + 5.0 gms. "	16 "	
16	" " " "	15 "	15.5 "
17	50 gms. " + 2.5 gms. "	16 "	
18	" " " "	15 "	15.5 "
19	" " + 5.0 gms. "	17 "	
20	" " " "	17 "	17 "
21	10 gms. " + 5.0 gms. $CaCO_3$.	16 "	
22	" " " "	18 "	17 "
23	20 gms. " + " "	23 "	
24	" " " "	16 "	19.5 "
25	50 gms. " " "	20 "	
26	" " " "	18 "	19 "
27	" $CaSO_4$.	16 "	
28	" "	16 "	16 "
29	" $CaCO_3$.	17 "	
30	" "	Lost "	17 "
31	2.0 gms. K_2SO_4.	24 "	
32	" "	23 "	23.5 "

The results of this experiment are not as clear cut as they should have been with larger yields of dry matter. As they stand, the influence of marl as a source of potash is not apparent, when it was used alone or together with gypsum. On the other hand, we observe that in boxes 21 to 26, where the marl was used together with calcium carbonate, there was an evident tendency towards the production of more dry matter. Further experiments to be carried out on a larger scale may show, therefore, that in the presence of large amounts of carbonate of lime marl may prove an effective source of potash in localities where economic conditions may permit its use.

SOME BACTERIOLOGICAL RELATIONS IN SOILS KEPT UNDER GREENHOUSE CONDITIONS.

SOME BACTERIOLOGICAL RELATIONS IN SOILS KEPT UNDER GREENHOUSE CONDITIONS.

Soils in the greenhouse are exposed to conditions that are admittedly more or less artificial. The range of temperature and moisture is not the same as that in field soils, while the aeration of greenhouse soils is, if anything, even more artificial. The artificial conditions are emphasized still more strongly when the soil is kept in small pots. The operations incident to the filling of the pots and the applications of fertilizers involve a more intimate contact of the soil particles with atmospheric oxygen than is possible under field or garden conditions. This leads to an abnormal multiplication of the soil bacteria and to a consequent abnormally rapid oxidation of the organic matter. In the course of time the more readily decomposable portions of the organic matter in the soil become depleted and this is followed, in turn, by a decline in the numbers of bacteria that will grow on agar plates. It is possible that the rapid falling off in numbers is due not merely to the depletion of the readily decomposable organic matter, but also to the accumulation of certain cleavage products injurious to the bacteria. Of the latter factor we have no direct knowledge and it is referred to in this place only as a possible explanation of the facts recorded below. It may be added here, also, that the greatly decreased number of bacteria appearing on agar plates should not be accepted as absolute proof that the total number of microorganisms in the soil had diminished. There is a possibility that a compensating increase had occurred of bacteria that do not grow on agar plates, as for instance, the nitrous and nitric ferments, &c.

With the foregoing observations as an introduction we may now turn to the examination of some data concerning quantitative bacteriological relations in soils kept under greenhouse conditions. The experiments under consideration were carried out either in earthenware or glass pots each containing twenty pounds of soil.

The various soil portions received different chemical or bacterial treatment, since it was the purpose of the experiments to determine whether such treatment would affect the numbers of bacteria producing colonies on agar plates.

Series I.

THE EFFECT OF VARYING QUANTITIES OF ACID PHOSPHATE AND OF CITRIC ACID ON THE NUMBER OF COLONIES ON AGAR PLATES.

Fourteen portions of fertile soil were placed in large, tared glass pots and maintained at a water content of 14 per cent. No crop was grown on these soils. The several soil portions were treated as follows:

Pot No.	*Material Applied.*	*Colonies on Agar Plates Per Gram of Soil.* *Dec. 10.*	*Dec. 21.*	*Jan. 14.*	*Feb. 4.*
1	18.14 gms. carbonate of lime.	6,000,000	6,200,000	4,000,000	3,200,000
2	0	7,920,000	6,000,000	4,460,000	2,600,000
3	0.1496 gms. acid phosphate.	6,000,000	7,800,000	4,240,000	2,640,000
4	0.2992 " " "	4,600,000	7,300,000	3,900,000	2,280,000
5	0.5984 " " "	3,640,000	4,720,000	3,400,000	2,180,000
6	1.1968 " " "	7,600,000	7,400,000	4,140,000	2,640,000
7	1.7952 " " "	4,480,000	5,340,000	4,400,000	2,840,000
8	18.14 gms. carbonate of lime.	6,400,000	7,840,000	3,700,000	2,860,000
9	0	7,120,000	7,800,000	3,900,000	2,800,000
10	0.3233 gms. citric acid.	6,840,000	6,400,000	4,600,000	3,000,000
11	0.6466 " " "	9,400,000	5,500,000	4,040,000	3,300,000
12	1.2932 " " "	14,400,000	13,000,000	4,700,000	3,920,000
13	2.5864 " " "	11,400,000	9,230,000	5,100,000	3,320,000
14	3.8796 " " "	12,600,000	10,300,000	7,200,000	3,680,000

The experiment was begun on November 17th, and about three weeks later the first samples of soil were drawn for quantitative examination. The soil suspensions for plating were prepared by shaking 100 gms. of soil with 200 c.c. of sterile water and making the proper dilution for inoculation into synthetic agar.[1] The plates were kept in the incubator at 28° C. and counts were made at the end of three days. Plates were similarly prepared from samples drawn on December 21st, January 14th and February 4th.

In comparing the results secured with the first set of samples we observe that the acid phosphate caused a gradual decrease in

[1] N. J. Sta. Rep., 1908, p. 133.

numbers when 0.1496 gms., 0.2992 gms. and 0.5984 gms. were used. When the amount of acid phosphate was further increased to 1.1968 gms., there was an increase, rather than a decrease, in the number of colonies. A still further increase in the amount of acid phosphate used was again followed by a decrease. It seems, also, that the application of lime depressed the number, since pots 2 and 9 to which nothing was applied, contained 7,920,000 and 7,120,000 bacteria, respectively, per gram of soil; while pots 1 and 8, that had received additions of carbonate of lime, contained only 6,000,000 and 6,400,000 bacteria, respectively, per gram of soil. We observe, likewise, that the addition of citric acid stimulated the multiplication of the bacteria three weeks after the beginning of the experiment. With 0.3233 gms., 0.6466 gms. and 1.2932 gms. of citric acid the number of bacteria per gram of soil were 6,840,000, 9,400,000 and 14,400,000, respectively. Beyond that the increased application of citric acid led to an increase and then to a decrease in the number of bacteria.

On examining the results secured with the samples of December 21st, we note corresponding relations in most instances. The applications of citric acid still showed their influence in the increased number of bacteria in most of the soils, while the acid phosphate showed an increase in some cases and a decrease in others. On the whole, however, we note a tendency toward smaller numbers in the soils that had received additions of citric acid, and a tendency towards greater numbers in the soils that had received additions of acid phosphate.

In the samples of January 14th we already observe not only smaller numbers of bacteria in all cases, but a distinct tendency towards the obliteration of the differences noted in the samples of December 10th and December 21st. Nevertheless, we can still detect even here the depressing effect of some of the smaller applications of acid phosphate, and the stimulating effect on bacterial growth, of the applications of citric acid. Thus the minimum number of bacteria was found in soil 4 that had received an application of 0.5984 gms. of acid phosphate, and the maximum number in soil 14 that had received an application of 3.8796 gms. of citric acid.

Finally, in the samples of February 4th we find a still more marked decline in numbers and pronounced tendency towards the establishment of uniformity in so far as the number of colonies on

agar plates was concerned. The influence of the applications of acid phosphate is scarcely apparent here, while the influence of the applications of citric acid is shown to a comparatively slight extent. There is no doubt, therefore, that in the interval between December 21st and February 4th the numbers of bacteria in all of the soils under experiment decreased very rapidly either on account of the depletion of the available organic nutrients, or on account of the accumulation of injurious cleavage products.

Series II.

Influence of small additions of fertile soil on the bacterial content of quartz sand properly supplied with plant-food.

The present series consisted of eight tared glass pots each containing 19.8 pounds quartz sand and 0.2 pounds fertile soil. In some instances the fertile soil was sterilized previous to the mixing with the sand. Four of the soils were to be seeded to oats, the other four, exactly alike in treatment, were to remain uncropped. The fertilizer additions to each soil consisted of:

4.0	gms.	acid phosphate,
2.0	"	muriate of potash,
0.5	"	magnesium sulphate,
0.01	"	ferric sulphate,
2.0	"	dried blood,
5.0	"	ground oyster shell lime.

The special treatment of the soils was as follows:

Pot No.			
1	Unsterilized soil,	oats.	
2	"	"	"
3	"	"	uncropped.
4	"	"	"
5	Sterilized soil,	oats.	
6	"	"	"
7	"	"	uncropped.
8	"	"	"

The oats were seeded on December 2d and harvested on April 2d. They were found to contain the following amounts of dry matter and of nitrogen:

Pot No.	*Dry Matter. gms.*	*Nitrogen. %*	*Nitrogen. mgs.*	*Average. mgs.*
1	6.5	1.19	7.73	
2	5.2	1.42	7.38	7.55
5	9.2	1.51	13.89	
6	5.0	1.52	7.60	10.74

In pot 5 that had received an addition of sterilized soil the yield of dry matter and of nitrogen was considerably larger than that in the corresponding pot 6. It will be noted, also, that the sterilization of the soil led to a larger proportion of nitrogen in the dry matter. This is not at all strange, for the sterilization of the soil in the autoclave causes the unlocking of some of the inert nitrogen compounds.

Bacterial counts were made in samples drawn December 15th, January 4th and February 25th. Synthetic agar was used for plating and the colonies were counted at the end of two days. Check counts made at the end of four days revealed larger numbers, but the results given here are those obtained at the end of two days. They are strictly relative and agree with the check counts. The numbers of bacteria per gram of soil as found at the different dates were as follows:

Pot No.	*December 15.*	*January 4.*	*February 25.*
3	7,000,000	3,700,000	840,000
4	9,400,000	3,480,000	780,000
7	3,780,000	7,000,000	1,200,000
8	4,420,000	8,500,000	1,178,000

It is apparent from the counts recorded above that the unsterilized soil caused a more rapid growth of the micro-organisms at the beginning. About two weeks after the initiation of the experiment, pots 3 and 4 contained, on the average, more than eight million of bacteria per gram of soil, while the corresponding pots 7 and 8 contained slightly more than four millions in the same quantity of soil. But in the samples of January 4th we find these relations reversed. The soils of pots 3 and 4 contained, then, about 3.5 millions bacteria per gram, while those of pots 7 and 8 contained 7.75 millions per gram. In the samples of February

25th the numbers had declined to a very marked extent, but the soils of pots 7 and 8 still contained a larger number of organisms than those of pots 3 and 4. We find, thus, that the sterilization of the soil, by eliminating the bacteria, furnished less favorable bacteriological conditions for the decomposition of the organic matter. However, this held good only for a short time. After that there was a more intense development of the micro-organisms, a more rapid decomposition of the organic matter favored by its exposure to a higher temperature and pressure in the autoclave, and a more abundant supply of available nitrogen compounds to the crop.

Later still there was a falling off in numbers both in the unsterilized and sterilized soil. In the samples of February 25th, the numbers were reduced to less than one million per gram of soil in pots 3 and 4, and to slightly more than one million in pots 7 and 8. Evidently the mineralization of the organic matter was proceeding rapidly and the numbers were decreasing with the decreasing supply of available food. Hence, the results of the present series agree completely with those of the preceding series.

Series III.

The numbers of bacteria in greenhouse soils as affected by additions of organic matter and of cultures of B. mycoides.

Like the preceding, this series consisted of eight tared glass pots. Four of them were filled with twenty pounds of quartz sand, and the other four with twenty pounds of fertile soil. The quartz sand received in each case additions of

4.0 gms. acid phosphate.
2.0 " muriate of potash.
0.5 " magnesium sulphate.
0.01 " ferrid sulphate.
2.0 " dried blood.
5.0 " ground oyster shell lime.

The fertile soil received additions of 5.0 gms. of oyster shell lime only. Aside from the materials enumerated some of the pots received additions of 0.2 pounds of green grass, and unsterilized or sterilized cultures of *B. mycoides*. Twenty-five cubic centi-

meters of a three-days-old culture of the latter was employed in each case. The various additions are shown in the following table:

	Pot No.		
Quartz sand...	1—Fertilizer, lime.		Sterile culture *B. mycoides*.
	2— " "		Live " "
	3— " "	green grass.	Sterile " "
	4— " "	" "	Live " "
Fertile soil....	5—Lime.		Sterile " "
	6— "		Live " "
	7— "	green grass.	Sterile " "
	8— " "	"	Live " "

The experiment was begun on December 8th, and samples for plating were drawn on January 7th, February 2d, March 5th and March 29th. Synthetic agar was employed as in the preceding experiments. The plates were kept in the incubator at 28° C., and their colonies counted at the end of three days. The numbers found per gram of soil were as follows:

	DATES OF SAMPLING.			
Pot No.	*Jan. 7.*	*Feb. 2.*	*March 5.*	*March 29.*
1.....	4,000,000	240,000	1,200,000	34,000
2.....	4,480,000	280,000	1,100,000	56,000
3.....	6,400,000	320,000	1,000,000	30,000
4.....	4,960,000	260,000	1,060,000	52,000
5.....	2,000,000	4,520,000	4,400,000	800,000
6.....	2,440,000	2,000,000	4,600,000	980,000
7.....	6,120,000	1,640,000	5,200,000	1,280,000
8.....	4,000,000	1,460,000	6,000,000	840,000

The samples of January 7th show the effect of the grass in both the quartz sand and in the fertile soil. In the former, the average number of bacteria per gram was 4,240,000, where no grass was used, and 5,680,000 where grass was used. The corresponding numbers in the fertile soil were 2,220,000 and 5,060,000. Aside from the influence of the grass we note that the additions of live cultures of *B. mycoides* increased the numbers in both the quartz sand and the fertile soil when no grass was employed, and decreased the numbers when grass was used. It is also interesting to note that at this time the quartz sand contained a greater number of bacteria than were present in the fertile soil.

On February 2d the quartz sand had become very poor in bacteria. The average number at that time was less than 300,000

per gram, and the influence of the additions of grass was scarcely apparent. In the fertile soil, on the other hand, the numbers of bacteria were maintained at a higher level. In fact, in pots 5 and 6 where no additions of grass were made, the average number of bacteria was greater on February 2d than it was on January 7th. In pots 7 and 8, on the contrary, there was a very marked falling off in numbers from the earlier to the later date. We may observe, likewise, that the soils of pots 3, 5 and 7, which had received additions of sterile cultures of *B. mycoides,* contained larger numbers of bacteria than the corresponding soils 4, 6 and 8 that had received cultures of live bacteria.

When we compare the numbers found in the different soils on March 5th we observe, in the first place, an appreciable increase in all but one case. The increase is particularly marked in the quartz sand where the numbers rose from less than 300,000 to more than 1,000,000 per gram. An even larger increase occurred also in soils 7 and 8 where the corresponding numbers rose from a little over 1.5 millions to more than 5.5 millions. It seems, also, that the soils which had received additions of live cultures of *B. mycoides* showed a tendency towards greater numbers than those present in the corresponding soils that had received additions of sterilized cultures. This is contrary to the results secured with the samples of February 2d.

The samples drawn on March 29th contained much smaller numbers of bacteria than those present in the other samples. In the quartz sand the numbers were reduced, on the average, to less than 50,000 per gram, while in the soil they were reduced to less than 1,000,000 per gram. The influence of the additions of grass was no longer marked at this date. It appears, therefore, that in the quartz sand the additions of live cultures of *B. mycoides* (pots 2 and 4) was not without influence on the numbers found on March 29th. Generally speaking, therefore, we observe in this as in the preceding series an unmistakable decline in the numbers of bacteria in the soil, or at least of those bacteria that form colonies on agar plates under aerobic conditions.

Series IV.

THE INFLUENCE OF GYPSUM ON THE NUMBERS OF SOIL BACTERIA THAT FORM COLONIES ON AGAR PLATES.

Twelve portions of fertile soil each weighing eighteen pounds were placed in earthenware pots, and treated as follows:

Pot No.	*Treatment.*	*Yield of Dry Matter.*	*Average.*
1	0	16.2 gms.	
2	0	6.2 "	11.2 gms.
3	2 gms. gypsum.	17.7 "	
4	" "	5.2 "	11.4 "
5	4 gms. "	10.8 "	
6	" "	6.3 "	8.5 "
7	8 gms. "	20.2 "	
8	" "	10.7 "	15.4 "
9	0	.....	
10	2 gms. gypsum.	.....	
11	4 " "	.....	
12	8 " "	.....	

The soil in pots 1, 3, 5 and 7 was seeded with vetch; that in pots 2, 4, 6 and 8 with oats. The soils in pots 9 to 12 remained uncropped and were sampled from time to time for bacteriological examination.

In looking over the yields of dry matter as given in the foregoing table we find that the two gms. of gypsum produced no appreciable increase or decrease. The four gms. of gypsum apparently reduced the yields, but on closer examination we find that the decrease occurred in the vetch and not in the oats. On the other hand, the eight gms. of gypsum produced an unmistakable increase in both the vetch and the oats. There is reason to think, therefore, that the applications of gypsum were not injurious to the soil bacteria, nor to the plants themselves. The larger yields of dry matter in the soils that had received additions of eight gms. of gypsum may be partly, if not wholly, accounted for by the larger amounts of nitrate furnished to the plants in these soils. This is evidenced by the nitrate determinations made on March 1st and April 26th. The following amounts were found: